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BIOLOGICAL TREATMENT OF CYANIDE WASTES

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BIOLOGICAL TREATMENT OF CYANIDE WASTES

by
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ABSTRACT

Degradation of cyanide by the complete-mixing activated sludge process of biological waste treatment was investigated in a laboratory pilot plant to determine the feasibility and economy of this process for treating industrial cyanide wastes, and to obtain scientific and engineering data for the design of a prototype plant.

Potassium cyanide was fed into the system, with ammonium acetate and a synthetic sewage as supplemental feeds. As a check on the possible influence of solids age, both sludge wasting (young cells) and extended aeration (old cells) were studied. In both cases, with the synthetic sewage supplement the system degraded all cyanide applied at and below a loading rate of 5 mg CN^- per gram of mixed-liquor volatile solids per hour, but sludge wasting was more reliable than extended aeration. Nitrogen balances indicated that the cyanide nitrogen is converted to nitrates. Biochemical analysis employing a radioactive tracer showed that two-thirds of the cyanide carbon is incorporated into cellular material and one-third is used for cell respiration.

Construction and operating costs for a full-scale complete-mixing activated sludge treatment plant were estimated to be about 50% of comparable costs for alkaline chlorination, the usual chemical treatment.

PREFACE

The content of Engineering Research Bulletin B-88 is essentially the same as the final report on a sponsored research project titled "Aerobic Metabolism of Cyanogenic Compounds," submitted in September 1963 to the Division of Water Supply and Pollution Control, United States Public Health Service. The work here described was supported by Research Grant WP-00151-04, and was performed under my supervision by R. Sage Murphy, a doctoral candidate in the Department of Civil Engineering, The Pennsylvania State University. His dissertation, dated December 1963, bears the title "The Activated Sludge Assimilation of the Cyanide Ion," but otherwise duplicates the project final report.

JOHN B. NESBITT

University Park, Pennsylvania
June 15, 1964

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1. SUMMARY

All industrial cyanide waste treatment facilities now use chemical methods, the most prevalent being alkaline chlorination. Some of the microorganisms normal to biological waste treatment are capable of converting cyanide to harmless carbon and nitrogen compounds. Since biological waste treatment is usually less expensive than chemical treatment, this suggests a more economical means of degrading the cyanide in industrial wastes. The purposes of the research here reported were to determine the cyanide assimilation capacity of an activated sludge system and the economic feasibility of biological cyanide treatment.

A bench-scale complete-mixing activated sludge unit was operated continuously for over two and one-half years to obtain the scientific and engineering data necessary to design and operate a prototype facility. Since previous work had indicated that a source of organic carbon other than the cyanide ion is required for cell synthesis, ammonium acetate and a synthetic sewage were tried as supplementary feeds. The synthetic sewage supplement was found to be superior and was used for most of the work.

Sludge-wasting and extended-aeration studies were made with the synthetic sewage supplement, to check any influence of the age of the cells. In both studies, the maximum cyanide loading rate was about 5 mg CN^- per gram of mixed-liquor volatile solids per hour, with 99% or more cyanide removal. Sludge wasting was recommended for the prototype plant, however, because the sudden loss of solids that is characteristic of extended aeration may cause the system to fail.

Nitrogen balances showed that the cyanide nitrogen converts quantitatively to nitrate nitrogen. Biochemical tests employing $KC^{14}N$ as a tracer demonstrated that under equilibrium conditions one-third of the cyanide carbon is used by the biota for respiration and the remaining two-thirds for cell synthesis.

An economic projection based on the data from this research gave biological treatment a decided advantage over chemical treatment. For hypothetical plants of each type, treating a typical waste containing the cyanide ion, estimated costs were $0.73 per pound of CN^- removed by the biological process, $1.44 per pound by alkaline chlorination. These figures include construction and operating costs, but not the cost of labor and site, which would be about the same for both processes.

2. INTRODUCTION

PURPOSE AND SCOPE

Industrial wastes containing cyanide are a major problem throughout the industrialized areas of the world. Though the volume of cyanide waste is comparatively small, its toxicity to living organisms is greater than that of most other industrial wastes.

The common treatment for cyanide wastes is the chemical process of alkaline chlorination. Where a choice can be made, biological waste treatment is usually less expensive than chemical processes. A few species of bacteria have been shown to be capable of metabolizing the cyanide ion to harmless carbon and nitrogen compounds (19,29,34),* implying the possibility of biological treatment. The objectives of our research were to investigate that possibility in a laboratory pilot plant, to develop engineering criteria for a full-scale prototype facility, and to determine whether biological treatment of industrial cyanide wastes would be more economical than alkaline chlorination.

GENERAL PLAN

The several aspects of this research, including a survey of the cyanide problem, are reported in detail in the sections that follow.

The bench-scale pilot plant was a complete-mixing activated sludge unit. In this unit cyanide-metabolizing bacteria and potassium cyanide were utilized to determine the capacity of the system to degrade cyanide biologically. Because previous work had indicated that a supplemental carbon source is needed for cell synthesis, separate experiments were conducted with ammonium acetate and a synthetic sewage as supplemental feeds. With the synthetic sewage supplement, both conventional sludge wasting (young cells) and extended aeration (old cells) were tested to reveal any effect of the age of the cells. The system was operated continuously for more than two and a half years.

Factors for the design of a full-scale prototype facility were developed from the pilot plant study and from biochemical tests, both of which included experiments with $KC^{14}N$ as a tracer.

The research data were applied to an engineering analysis of the feasibility of biological treatment for industrial cyanide wastes. Estimated costs for this method were compared with the known costs of alkaline chlorination, the usual chemical method, to measure the economic advantage of biological treatment.

TOXICITY OF THE CYANIDE ION

The Drinking Water Standards of the U.S. Public Health Service (2) recommend a limiting concentration of cyanide, expressed as HCN, of 0.01 mg per liter. In public water supplies, the mandatory limit is 0.02 mg/l. Hopkins and Gullans (13) state that the mandatory limit has a safety factor of approximately 100. With no factor of safety the limiting concentration would be 2.0 mg/l, a figure which clearly shows the extreme toxicity of the cyanide ion to human beings.

The biochemistry of the lethal effect of cyanide is complicated, but the general reaction can be described as follows. The cyanide ion forms a highly stable complex with cytochrome oxidase, an enzyme concerned with cellular oxidation, making the cytochrome oxidase inactive.

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*See References.

Death ensues because utilization of oxygen for an important metabolic reaction is prevented (12).

How lethal the cyanide ion is to man is uncertain because of the obvious restriction on experimental work, but it is known to be toxic in varying degrees, regardless of the radical of which it is a part. Direct injury to man, sickness or death, is the most feared consequence of allowing cyanide to leave an industrial plant untreated, though instances of such injury are rare. As is true of most other wastes, the indirect consequences are of greater economic, hygienic, and aesthetic importance. These are depletion of wildlife, degradation of streams, and degeneration of the natural biological activity in controlled and uncontrolled environments. The cyanide radical should be eliminated immediately after it has performed its industrial or chemical duty and before it can be injurious to man either directly or indirectly.

The Drinking Water Standards of the Public Health Service relate to the continuous consumption of water by human beings. Limits for the protection of fish and other biota in receiving streams are governed by other criteria. The Ohio River Valley Sanitation Commission (4) considers free cyanide in excess of 0.025 mg CN^-/l to be unsafe for fish life in the waters of the Ohio River Basin. That limit was obtained by exposing different species of fish to variable concentrations of cyanide over extended periods of time. Schant (42) found that minnows, catfish, and carp exposed to sodium cyanide were not killed by 0.3 mg CN^-/l, but acted sick and then recovered in 24 hours. Potassium cyanide kills goldfish in 3 to 4 days at exposures of 0.04 to 0.12 mg CN^-/l (11). Many variables besides the cyanide ion concentration seem to affect fish life, the more important being pH, water temperature, oxygen concentration, and various impurities. Since all of these may change radically in a rather short reach of a stream, the lower experimental values for the cyanide concentration are generally used if fish life is important in the stream under consideration.

Cyanide toxicity data for other forms of life in streams are lacking in the literature. If only the toxicity to fish is considered, the limiting concentrations are not greatly in excess of the mandatory limit for drinking water. The concentrations reported for fish were those that actually caused the organism to become sick or die. Assuming that other life in a stream is similarly affected by cyanide, the stream will be degraded if any part of the biological cycle is removed for an extended period. Should the fish die because of cyanide poisoning, the immediate result would be decaying fish and a septic stream. Whether they die or leave, the stream is likely to be taken over by smaller organisms whose growth cannot be restrained without the presence of the higher forms of life. The ultimate consequence is both the economic loss of a water resource and the aesthetic loss of a part of nature.

Man-made biological environments such as sewage treatment plants are very easily upset by exposing the organisms to the cyanide ion. Although cyanide is amenable to aerobic biological degradation, all reports show a definite inhibition of the treatment process when the ion is applied to an unacclimated culture. If an aerobic unit has not been acclimated to cyanide, a concentration greater than 1 mg/l will

retard the process, producing an effluent of poor quality (7,17). That the effect is inhibitory rather than lethal is demonstrated by the recovery of trickling filters (35) and activated sludge plants (8,45) after an initial dose. Anaerobic treatment units appear to be much more sensitive to the presence of cyanide than aerobic units. Coburn (7) and Laboon (16) set the limit of cyanide at 2 mg/l for satisfactory anaerobic sludge digestion without a digester upset. No recovery of efficiency after prolonged exposure to cyanide has been reported for any anaerobic process, but aerobic processes invariably recover from initial cyanide loadings, with the benefit that much of the cyanide has been reduced biologically.

Unless a cyanide-bearing waste can be diluted to a high degree before reaching a treatment plant, so that it enters the plant without being a shock load, all cyanide must be removed from the waste before it is discharged into a sewer. When a treatment process is upset by cyanide, the effects on a stream may be much worse than the effect of cyanide alone. First, the plant effluent may contain enough cyanide to kill fish and other organisms; second, the effluent will contain only slightly less organic matter than raw sewage, nullifying the plant's function and grossly polluting the stream.

Regardless of the source, cyanide is a waste that has caused much economic loss, both directly and indirectly, when permitted to enter streams and treatment plants without proper dilution or pretreatment.

SOURCES OF CYANIDE WASTES

Cyanide wastes originate mainly in metal hardening, finishing, and plating operations, in coke and gas processing, and in the manufacture of chemicals, pharmaceuticals, plastics, and steel. Nearly every industrial plant that uses metal in its finished product has a shop with a cyanide waste problem. Metal hardening, finishing, and plating contribute the largest percentage. Though most of the plants specializing in these operations are small, there are many of them and they are situated in large industrial areas.

The wastes arise from the fact that before metals can be plated all undesirable foreign substances must be removed from their surfaces by a stripping procedure. Sodium cyanide is often used in this step. The actual plating process employs an electrolytic cell whose cathode is the metal to be plated and whose electrolytes are copper, cadmium, zinc, brass, or silver cyanide complexes. The cyanides enter the waste system from drag-out losses carried into subsequent rinsings, and from leakage and accidental spills of the plating tanks. The concentrations of the cyanide ion in the electroplating baths range from 20,000 to 60,000 mg CN^-/l. The quantity of solution dragged out of each bath is a function of the size and shape of the piece to be plated and the velocity with which it is withdrawn from the bath. The concentration of cyanide in the waste itself is governed by the same factors and the amount of water used in the rinse. No generalization can be made concerning the volume or concentration of cyanide that can be expected from a specific plant. In Connecticut the volumes vary from 2000 to 350,000 gallons per day, and the CN^- concentrations vary from 100 mg/l to 50,000 mg/l, depending on the process and on efficiency and housekeeping at the plant (40).

The carbonization of coal in by-product coke-oven plants and city gasworks is another source of cyanide, but the latter are becoming obsolete as a result of the increasing use of natural gas. Most plants of this kind practice recovery operations that reduce the effluent cyanide concentration to about 10 mg/l (40). This source is more localized than metal finishing, and because of the shift to other types of energy its contribution is steadily decreasing.

Cyanide wastes from other sources are comparatively small, or they are organic cyanides from the pharmaceutical, synthetic fiber, and plastics industries.

3. TREATMENT OF CYANIDE WASTES

CHEMICAL METHODS

Present treatments for the removal of cyanide from industrial wastes are either chemical or physical processes or a combination of the two. The most satisfactory method and the one that predominates in the United States is the chemical process of alkaline chlorination. None of the methods now practiced is without disadvantage, but chlorination appears to have only one serious drawback: if too much chlorine is used, the chlorinated effluent may be just as toxic in a stream as untreated cyanide. This can be prevented, of course, by good plant control.

The exact nature of the alkaline chlorination reaction has not been formulated, but the general reaction is

$$2NaCN + 8NaOH + 5Cl_2 \longrightarrow 10NaCl + 2CO_2\uparrow + N_2\uparrow + 4H_2O$$

with indications that cyanogen chloride is formed as an intermediate. Some investigators believe ammonia is an end product, a result which is not likely because ammonia reacts with chlorine, forming chloramines and finally nitrogen.

The evidence that the reaction does resolve to harmless end products is sufficient to permit an estimate of the chemicals needed to treat a cyanide waste. The theoretical requirement for the overall reaction is 6.8 parts of chlorine and 6.2 parts of sodium hydroxide per part of cyanide. A high pH is required at all times during the process to prevent hydrogen cyanide evolution and the formation of nitrogen trichloride. Values of 8.5 to 11.0 have been most frequently mentioned for the pH range. Detention times in batch plants range from 20 min to 24 hr. When free available chlorine is present, the cyanide ion is completely removed if the plant effluent remains above 8.5. Many plants use the alkaline effluent to neutralize acid wastes from other processes.

Acidification has been practiced by plants ranging in size from small two-man shops to industrial giants such as the Kaiser-Frazer Corporation at Willow Run, Michigan. A cheap acid or waste acid is used for the reaction

$$2NaCN + H_2SO_4 \longrightarrow 2HCN\uparrow + Na_2SO_4$$

This method has two disadvantages. One is the very noxious hydrogen cyanide gas it produces. Most installations discharge the gas through tall stacks with a high ratio of air dilution, relying upon the exit velocity from the stack to disperse the HCN so that its concentration in local areas never rises above toxic levels. The second disadvantage is a residual cyanide concentration of about 1 mg/l in the liquid, which must be disposed of by other methods, usually dilution in an adjacent watercourse.

A similar but less controlled method of treatment is ponding. The rate at which cyanide is removed depends almost entirely upon pH. A waste containing 100 mg CN^-/l was reduced to 5 mg/l in 48 hr at pH 3.0 in a pond 2 ft deep (9). The disadvantages of ponding are the large land areas required, the risk of polluting ground waters, the danger of dike breaks, and the level of residual cyanide after the detention period.

Complexation of the cyanide ion into very stable, less toxic compounds has been used as a treatment method. A typical reaction is

$$Fe^{++} + 2OH^- \rightleftharpoons Fe(OH)_2$$

$$Fe(OH)_2 + 6CN^- \rightleftharpoons Fe(CN)_6^{-4} + 2OH^-$$

The equilibrium will shift to the left if more base enters the system or is found in the ultimate disposal area, thus freeing the cyanide from the complex. Complexation is only an intermediate step, as the sludge formed in the process must itself be disposed of in a suitable place. Treatment efficiency is about 90%, which is usually insufficient for liquid effluent discharged into a sewer or a watercourse.

Electrolytic oxidation of the cyanide ion to cyanates has been known and used by industry for many years. The process employs normal plating-room equipment, with copper electrodes in a concentrated bath. The advantages of possible metal reclamation, no sludge disposal problems, and familiar equipment are generally thought to be overshadowed by the disadvantages of concentrated solutions, excessive time requirements, and cyanate end products that may require further treatment before being discharged.

Ozonation has been studied on a laboratory scale (46) as a possible method of treating cyanide. The general reaction is

$$2KCN + H_2O + 5O_3 \longrightarrow 2KHCO_3 + N_2\uparrow + 5O_2\uparrow$$

The ozone must be applied to a solution with a pH of 11.0 to 12.0 until free ozone appears in the effluent gases. Like chlorination, this method goes through cyanate before it forms bicarbonate and nitrogen gas. The process shows promise, but no full-scale treatment units have been reported.

BIOLOGICAL DEGRADATION

The principal disadvantage of all of the chemical methods just described is their cost. It is the aim of the present research to show that biological degradation of the cyanide in industrial wastes is a practicable and much less expensive alternative. Some of the microorganisms active in biological waste treatment are known to be able to metabolize the cyanide ion to harmless carbon and nitrogen compounds. Both the mechanism and the extent of this capability have been explored by a number of workers.

The possibility of using a conventional biological waste treatment plant for the removal of cyanide was first recognized by Pettet and Mills (34), who used a trickling filter. Cyanide interfered with the treatment efficiency when first applied to the filters at concentrations of 1 to 2 mg/l, but the efficiency of the filter returned to normal in a week. The cyanide was added to the filter mixed with sewage which the filter was treating previously. The acclimated filter treated 100 mg/l, as HCN, with 99% or greater destruction of the cyanide ion.

Painter and Ware (32), working with the biota developed on the filter by Pettet and Mills, isolated an organism that grew on a silica gel medium containing potassium cyanide as the only source of carbon and nitrogen. The isolated bacterium was provisionally classed among the Actinomycetaceae. The organism was aerobic, a strict autotroph, grew very slowly, and lived optimally within a pH range of 7.5 to 9.0.

The British Water Pollution Research Laboratory (10) utilized the activated sludge process in studies of cyanide destruction. The system was operated on the fill-and-draw basis, with a total liquid volume of about 200 liters. The cyanide concentrations ranged from an initial value of 1 mg/l to a maximum of 180 mg/l, with greater than 99% removal of the cyanide at all times. Recovered ammonia nitrogen varied from 3 mg/l to 90 mg/l as the cyanide load increased, and the oxidized nitrogen forms varied from 13 mg/l to 6 mg/l during the same period.

Ludzack and others (21) studied the biochemical oxidation of a few commercially important organic cyanides in an aerated surface water. The nitriles studied were acetonitrile, acrylonitrile, adiponitrile, lactonitrile, and oxydipropionitrile. The fact that they are different chemically was important to the study, although they do not represent all types of nitriles found in wastewaters.

This study, designed to determine the natural purification characteristics of these nitriles in surface waters, showed that all the nitriles were amenable to biological degradation in varying degrees. An acclimated biota was able to metabolize a given nitrile in spite of wide variations in concentration and feeding intervals. Each nitrile-degrading biota was quite specific for the nitrile to which it was acclimated, and less effective in stabilizing the more resistant forms.

Organisms adaptable to nitrile oxidation were found to be common in the surface water tested (Ohio River water obtained at the intake to the Cincinnati Water Works). Certain of the nitriles required additional

nutrients, such as domestic sewage, to maintain active growth in the laboratory system. The organic cyanide concentrations used for the various tests ranged from 0.1 to 25 mg/1.

Ludzack extended the work on the nitriles from surface water studies to treatability by conventional sewage disposal processes (20). Because of the high microbial populations in activated sludge, these sludges required relatively short acclimation times to achieve a high treatment efficiency. Those acclimated on one nitrile had similar degradation characteristics when they were used on the other nitriles. Nitriles were shown to have a definite inhibitory effect on anaerobic decomposition.

Nesbitt and others (29) studied the aerobic metabolism of cyanide compounds in an activated sludge system in which no mixed-liquor suspended solids were wasted and in which cyanide served as the only source of carbon and nitrogen. The system used in the study was fed potassium cyanide at both 1 hr and 1/2 hr intervals. The daily operating procedure consisted in stopping the feed and air supply and allowing the mixed liquor to settle 1 hr, then decanting 1 liter of the settled liquor from a total of 3 liters and analyzing it for cyanide. The system was considered to be semicontinuous.

Results with this unit showed that the system metabolized 99^{+}% of the potassium cyanide. The maximum cyanide level attained was 120 mg CN^{-} per day, with greater than 99% removal. During the time the unit was fed 120 mg CN^{-}, the mixed-liquor suspended solids (MLSS) had a maximum value of 234 mg/1 and a minimum of 112 mg/1, with a median value of 154 mg/1. At this feed rate, the organisms in the unit were metabolizing cyanide at the rate of 10.5 mg CN^{-}/g MLSS/hr. Increasing the solids level in the mixed liquor was difficult because the predominant organism in the sludge was a very slow-growing one.

An additional semicontinuous system was developed with sodium acetate as a supplemental source of carbon. To the cyanide-acclimated mixed liquor, 4270 mg of acetate were added per day for the first 10 days, and the acetate was then reduced to 1780 mg per day. At the end of 25 days this unit attained an MLSS level of 1250 mg/1. The control unit remained at a constant solids level of about 250 mg/1. Both units were fed 120 mg CN^{-} per day throughout the test, and complete treatment of the cyanide was realized in each reactor. The respective loading rates for the supplemented unit and the control unit were 1.49 and 7.1 mg CN^{-} per gram of mixed-liquor volatile solids (MLVS) per hour.

A continuously fed extended-aeration system also was operated for approximately 30 days, using a cyanide feed supplemented with sodium acetate. Results were inconclusive because of the short observation time, but the ability of such a system to metabolize and remove cyanide from solution was demonstrated.

Ludzack and Schaffer (19) studied the activated sludge treatment of cyanide, cyanate, and thiocyanate. Their bench-scale extended-aeration system (18) was fed cyanide with a supplement of domestic sewage and dextrose. The three cyanide compounds were effectively degraded by activated sludge after two to three weeks of acclimation. Dextrose

hastened the acclimation time. Activated sludge treatment of cyanide was less effective above a feed concentration of about 60 mg CN^-/1. Above that concentration cyanide removal and nitrification decreased, but cyanide stripping by aeration increased. A cyanide concentration of 60 mg/1 in this system was equivalent to about 3.0 mg CN^-/g MLVS/hr, a loading rate considerably less than that achieved by Nesbitt.

Aaslestad (1) isolated a strain of Arthrobacter from the pilot plant used during the early stages of the present research. The maximum concentration of cyanide this culture could degrade was 60 mg/1. He postulated that cyanide can serve as a nitrogen source but not as a carbon source, and that ammonia inhibits a growing culture but not a resting culture. The organism could convert cyanide to thiocyanate when sulfur was present in the feed, but thiocyanate was not thought to be an intermediate in the cyanide degradation.

Porter and Dutch (37) treated a coke-oven wastewater containing phenol and cyanide in a plastic-packed trickling filter. The phenol-cyanide ratio was 4:3, and the carbon-nitrogen-phosphorus ratio was 20:2:1. Hydrogen sulfide, also present at a concentration of 3 mg/1, had no adverse effects upon the treatment of this industrial waste. The average removal of cyanide was greater than 94% and of phenol greater than 99%, the respective loadings of cyanide and phenol being about 16 and 20 lb/day/1000 cu ft of filter material. Pettet and Mills (34) treated 37 lb CN^-/day/1000 cu ft on a rock-filled trickling filter with cyanide and sewage alone. No attempt was made in either of these cases to determine the quantity of hydrogen cyanide gas blown from the system.

Nitrogen is one of the primary requirements of any biological waste treatment system. The effects of different nitrogen compounds and of various amounts of each were studied by Symons and others (44). They postulated that (a) all degradable nitrogen compounds release their nitrogen into solution during metabolism, (b) once in solution this nitrogen can be used for synthesis, and (c) the mechanism of biological nitrogen removal is the same regardless of the form of the nitrogen group attached to an organic molecule. The conversion is to an amino group, with hydrolysis of various intermediates from the molecule, depending upon the original form.

Howe and Howe (14) studied the biological decomposition of cyanides, intending to determine the end products. After 24 hr of gentle aeration, 73 to 76% of the cyanide added could be reduced, 34 to 43% of the reduced fraction being absorbed or stored in the sludge. Increased aeration was accompanied by greater cyanide reduction and less cyanide storage in the sludge. With the addition of cobalt chloride, vitamin B_{12} was formed as an end product, but no quantitative data were presented.

Trickling filtration of a plating waste was studied in a laboratory pilot plant by Brink and Thayer (5). Concentrations of cyanide as high as 150 mg/1 were fed into the system, in the presence of heavy metal ions. The metals caused some problems but were not toxic to the organisms on the filter. Tests indicated the need for a supplemental organic feed. Most of the zinc present in the waste was precipitated as the cyanide decomposed, whereas half of the copper remained in solution under identical conditions.

Using a batch-fed system operated at 600 mg CN^-/1, Rayand and Bizzini (38) found that cyanide levels decreased rapidly, with no observable bacterial growth or oxygen uptake. Bacteriological studies reported the isolation of organisms with the following characteristics: (a) a gram-positive coccus grew slowly and destroyed cyanide in a few days, (b) a gram-positive sporulated bacterium grew slightly faster, and (c) a mobile gram-negative Pseudomonas grew rapidly (1 to 5 hr).

EFFECTS OF HEAVY METALS

Industrial wastes containing cyanides usually contain heavy metals, which are often as toxic as cyanides to treatment plants and stream biota. Electroplating contributes most of the heavy metals as well as most of the cyanides in the industrial wastes of this country. The metals commonly found in waste streams from plating plants are chromium, copper, nickel, zinc, and cadmium. Normal waste concentrations range as follows (40): copper, 11 to 58 mg/1; hexavalent chromium, 32 to 204 mg/1; nickel, 19 to 32 mg/1; zinc, 39 to 82 mg/1.

Because many electroplating plants practice recovery of these metals from their waste streams as an economic measure, such concentrations are unusual in wastewaters except as the result of accidental spills. Cyanide treatment by either the present chemical or the proposed biological method may therefore be independent of heavy metal waste treatment. If heavy metal ions do appear in the waste, their significance to the biological treatment of cyanides lies in the possible adverse effects upon the activated sludge process when they occur as either small constant concentrations or slug doses.

The effects of chromium on activated sludge were studied by Moore and others (28), using conventional pilot plant equipment. Chromate had no adverse effects except when it was applied in a massive slug dose. Hexavalent chromium in concentrations greater than 5.0 mg/1 passed through the system in varying quantities, but the efficiency of the plant was undisturbed except for a short period when nitrification stopped. The nitrification always resumed, even at constant chromate concentrations of 50 mg/1, indicating that the nitrifying bacteria can become acclimated to the chromate.

Chromium retention in the system occurred mainly in the activated sludge solids. Primary effluent and return sludge were mixed in a separate reactor, to which no air was supplied. The chromate then acted as an oxygen source for the sludge. When the effluent from this unit was brought into the aeration tank, the ultimate result was the conversion of all the hexavalent chromium to the less toxic insoluble reduced state.

The effect of copper on aerobic treatment was reported by McDermott and others (24) for acclimated and unacclimated activated sludge systems. Removal of biochemical oxygen demand (BOD) was reduced 0.1 to 7% when copper was continuously present in concentrations ranging from 0.4 to 25 mg/1 in the raw sewage. Copper sulfate and a copper cyanide complex had about the same effects on acclimated cultures. Slug-dose tests showed the copper cyanide complex to have a much more severe effect than the copper sulfate upon the efficiency of an unacclimated culture. When acclimated systems were fed slug doses, both of the copper compounds

exerted the same effect. Cyanide acclimation was considered more important than copper acclimation in the slug-dose tests. During the periods of continuous feeding of the copper cyanide complex, all the cyanide was biologically removed from the system.

McDermott and others (23) also studied zinc in relation to the activated sludge process. Both zinc sulfate and a complexed zinc such as occurs in alkaline cyanide plating baths had about the same effect after the sludge was acclimated. Zinc levels greater than 2.5 mg/l but less than 10 mg/l had no significant effect on treatment efficiency.

The literature concerning toxic limits for the various heavy metals in biological treatment facilities shows little agreement. Factors contributing to the differences of results are dissimilar solution pH, chemical form of the metals, temperatures and test organisms, and the interference of other elements or compounds. The metal toxicities most frequently reported are, in descending order, zinc, hexavalent chromium, nickel, copper, and cadmium (33). Only zinc is considered more toxic than the cyanide ion. Anaerobic treatment processes are usually much more readily upset by cyanides and metals than are the aerobic units.

The present study is concerned primarily with the biological treatment of cyanides. Since most cyanide wastes do contain heavy metals, their effect upon cyanide degradation is important, although the metals themselves may have to be removed from the waste by other means before or after biological treatment. Indications from the available literature are that the metal concentrations normally found in plating wastes are within limits to which activated sludge can become acclimated.

4. EXPERIMENTAL EQUIPMENT AND ANALYTICAL PROCEDURES

COMPLETE-MIXING ACTIVATED SLUDGE SYSTEM

Research showing the cyanide ion to be amenable to aerobic degradation, reviewed in Section 3, was performed on trickling filters and on batch-fed and continuously fed activated sludge systems. For the present investigation a continuously fed activated sludge unit was selected because it was easily adaptable to the requirements of a bench-top laboratory pilot plant.

From several existing modifications of the activated sludge process we chose a complete-mixing activated sludge system, since complete mixing is known to be very effective in the biological treatment of industrial wastes (26). It almost instantaneously diffuses the wastes throughout the volume of the aeration tank, thereby exerting a uniform oxygen demand and promoting uniform biological growth throughout the active mass. Because of the immediate high dilution produced by complete-mixing, the aeration tank also operates as a surge tank, leveling out variations of the incoming raw wastes.

Three approaches are possible with complete-mixing activated sludge treatment: aeration only, unintentional sludge wasting in the effluent (extended aeration), and deliberate sludge wasting. Most of our research

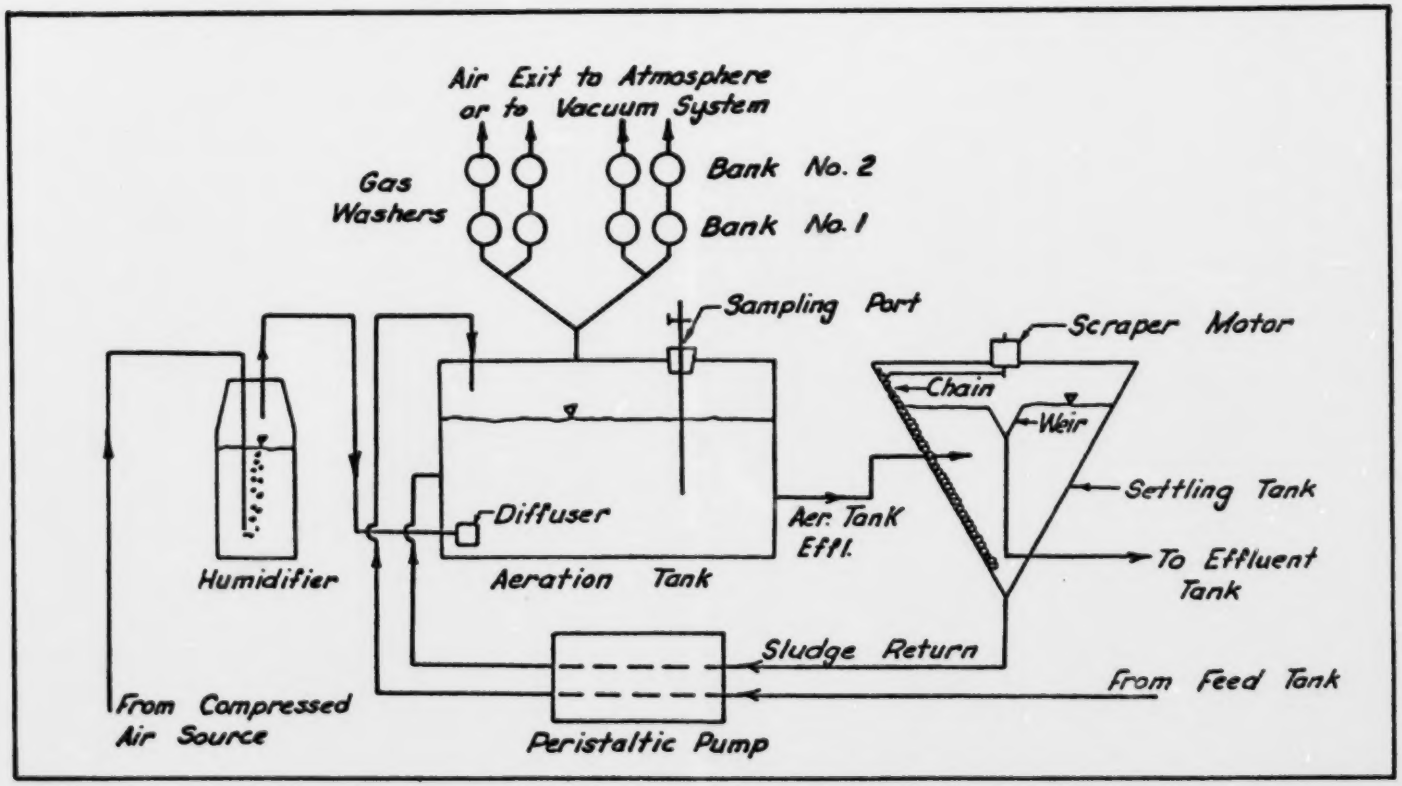


FIG. 1. Diagram of bench-scale complete-mixing activated sludge unit.

dealt with unintentional sludge wasting in the effluent, but one study was concerned with deliberate sludge wasting.

PILOT PLANT

Figure 1 is a schematic diagram of the complete-mixing activated sludge system used throughout the experimentation. The unit consisted of an aeration tank, a settling tank, an influent compressed-air system, an effluent air system, and a peristaltic pump.

The aeration tank, which was sealed to permit scrubbing of the exit gases, was constructed of 1.27 cm clear Lucite. The outside dimensions of the tank were 28 cm long, 18 cm wide, and 22.5 cm deep. Two fused crystalline alumina gas-diffuser stones, 2.54 cm long and 2.22 cm in diameter, were placed 1 cm off the bottom of the tank and spaced at the third points on the narrow side of the tank. Complete mixing was visually checked and found to exist when 0.283 cu m of air per hour was applied through the stones with 5.0 to 5.5 liters of liquor in the tank.

The influent feed was continuously applied through a tube that entered the aeration tank by a port directly above the diffuser stones. During part of the research the feed was allowed to drop into the mixed liquor, but the influent tube later was extended beneath the liquid surface to prevent possible cyanide losses induced by the air velocity above the liquid.

The aeration tank effluent line was located 8.5 cm above the bottom of the tank, in the center of the end opposite the diffuser stones. It was a Lucite tube, inside diameter 1.59 cm, set flush with the inner wall.

A 1.59 cm sludge return line from the settling tank was located 6 cm above the bottom of the tank, between the diffuser stones. This location allowed cells that had been in the unoxygenated settling tank to be exposed to the maximum oxygen concentration of the aeration tank immediately upon entrance.

A stopper with a glass tube through it was placed midway along the length of the aeration tank for mixed-liquor sampling. The glass tube extended to the approximate center of the activated sludge mass. On the outside of the tank a length of rubber tubing was attached to the glass tube to facilitate sampling. A hosecock was placed on the rubber tubing to prevent air from escaping from the aeration tank during normal operation.

A third port, through which all exit gases passed, was located on top of the tank. The effluent gas stream then passed through two banks of gas washers, each bank consisting of four washers. With sodium hydroxide in the washers the gases were analyzed for cyanide blown from the system, and with boric acid in the washers the gases were analyzed for ammonia blown from the system.

Two methods were used to collect the exit gases. Initially, the aeration tank was under pressure and the exit gases were forced through

the gas washers. The head required to pass the gases through the washers was equal to about 15 cm of water. Any change in head losses through the washers, or change in pressure of the influent compressed air, altered this head and caused difficulties in keeping the complete system in a state of equilibrium.

In the second method of collecting the effluent gases, a 0.3 cm air gap was left between the aeration tank port and a 2.5 cm cone attached to the effluent air line. An aspirator connected to the exit side of the gas washers created a vacuum sufficient to draw a quantity of air through the washers greater than the volume applied to the aeration tank. The efficiency of this method was easily verified by blowing smoke into the air gap and visually checking to see whether all the smoke was drawn into the washers. If the smoke was not drawn into the washers, this indicated that not all of the air leaving the aeration tank was going through the washers and a greater vacuum had to be applied. This method of collecting the gases eliminated the pressure in the aeration tank and therefore made the operation of the system more reliable.

The settling tank was a separate unit made of 0.318 cm Lucite. The unit was conically shaped, with sides at a 60 deg angle to the horizontal and the diameter changing uniformly from 25.4 cm at the top to 3.8 cm at the base. A 5 cm transition section below the 3.8 cm diameter uniformly took the apex of the cone to a nipple that accommodated 1.27 cm rubber tubing. The aeration tank effluent entered the settling tank through a tangential horizontal 1.59 cm tube located 10.2 cm below the top rim of the settling tank.

The settling tank effluent weir, located in the center of the tank and 3.0 cm below the upper rim, had a settling volume of 1.6 liters. Throughout part of the experimentation the weir was a 7.0 cm diameter funnel, but this was replaced with a piece of 1.27 cm glass tubing a week before the end of the acetate study. The vertical effluent tube was bent 90 deg at 6.4 cm above the bottom of the transition section and taken out through the side of the tank to a large carboy used to collect the effluent between sampling periods.

Supported over the settling tank was a clock motor with an arm the same length as the radius of the top of the tank. A chain, acting as scraper, was hung from the end of the bar to keep the sludge from accumulating on the sides. The scraper made one revolution per minute. The sludge that accumulated at the base of the tank was recirculated continuously to the aeration tank through a peristaltic pump operating at approximately 2 liters per hour.

Feed was made up to last two or three days, except that during the early stages of the work it was made for one day only, and stored in a large carboy. At low cyanide concentrations the carboy was kept in a cold water bath to inhibit decomposition of the feed, but at high cyanide levels this precaution was unnecessary because the cyanide prevented significant growths, as indicated by the turbidity of the solution.

The feed was transferred continuously to the aeration tank by the same peristaltic pump used for sludge recirculation. A tube of smaller

diameter was used for the feed, giving rates from 0.22 to 0.35 liter per hour, with 0.30 liter per hour as the average.

The compressed air used for aeration was brought through an oil trap, a pressure regulator, and a humidifier. The humidifier was a 10 liter carboy filled with water through which the air was bubbled, thus adding moisture to the air and eliminating the loss of liquid by evaporation in the aeration tank. The air went into the aeration tank immediately after humidification.

Air rates were periodically checked by a rotameter installed before the aeration tank, and by a wet test meter on the effluent air line during periods when the system was under pressure.

ANALYTICAL TESTS

Chemical Oxygen Demand. The chemical oxygen demand test was the 15-min test developed at the University of California (41,47).

Cyanide. Cyanide was determined by the silver nitrate titration procedure outlined in Standard Methods for the Examination of Water and Wastewater (3). To eliminate interferences the preliminary screening procedure was used on the samples. But if no difference was found between the screened and unscreened samples, the titration was made directly. The pyridine colorimetric procedure (3) was used a number of times on a purely qualitative basis to ascertain if cyanide was present in samples at concentrations below the sensitivity of the titration method.

Nitrogen, Ammonia. Ammonia nitrogen was determined by the distillation method outlined in Standard Methods.

Nitrogen, Nitrate. The phenoldisulfonic acid method for nitrate nitrogen (3) was used. All samples were pretreated with aluminum hydroxide cream and filtered for removal of color and suspended materials. A spectrophotometer with 4 cm cells was used for the colorimetric determinations.

Nitrogen, Nitrite. The method outlined in Standard Methods was used for the nitrite nitrogen determinations, with the same pretreatment method and spectrophotometer as in the nitrate test.

Nitrogen, Organic. Organic nitrogen was not determined directly, but was calculated from the difference between the total nitrogen and ammonia nitrogen determinations.

Nitrogen, Total. The Kjeldahl total nitrogen method was used as specified in Standard Methods.

pH: pH was determined electrometrically with a pH meter.

Solids, Suspended. The following procedure was used to determine suspended solids.

(1) The samples were transferred to 50 ml polypropylene centrifuge tubes, 40 ml of sample per tube. If the volume of the sample was not an even multiple of 40, the needed volume was made up with distilled water. The tubes reclined at a fixed angle of 34 deg. The centrifuge was run at a speed of 7200 rpm for 15 min, and the relative centrifugal force exerted was 6620 kg per gram. Centrifugation was used only as a means of consolidating the solids to facilitate the use of larger samples.

(2) A desiccated fine borosilicate filter paper, 5.5 cm in diameter, was weighed. Since the filter material was nonhygroscopic, predrying was unnecessary (30).

(3) The filter paper was placed, rough side up, in a standard filter assembly, suction was applied, and the centrifuged sample was poured onto the filter. After the supernatant from the tubes had been filtered, the solid mass was poured onto the filter and washed with distilled water.

(4) When the liquid had passed through the filter, the paper and residue were dried in an oven for a minimum of 2 hr, at 103° C.

(5) The dried samples were immediately weighed. The mass of the glass filter medium was so small that the dried samples could be placed on the balance without precooling in a desiccator (30).

(6) The weight of the suspended solids was the difference between the weight of the dried sample and that of the filter material. The concentration in mg/l is equal to

$$\frac{(\text{Wt. of sampled solids, mg}) \times 1000}{\text{Sample size, ml}}$$

Solids, Volatile. Volatile solids were determined by the following procedure.

(1) In a porcelain evaporating dish the filter paper and solids from the suspended solids determination were heated with a Fisher burner until the sample stopped smoking. The dish and solids were then heated at 600° C in a muffle furnace for 10 min. The preheating step was necessary to avoid flash ignition of the sample when placed in the furnace.

(2) The ignited residue was weighed.

(3) The weight of the volatile solids was the difference between the weight of this residue and the weight of the suspended solids and the filter. Any loss due to ignition of the filter was negligible. The concentration of volatile solids in mg/l is equal to

$$\frac{(\text{Loss on ignition, mg}) \times 1000}{\text{Sample size, ml}}$$

Other Tests. The analytical tests, as well as the experimental procedure and equipment, for the radioactive tracer study are discussed in Section 7.

5. ACETATE SUPPLEMENT STUDY

PILOT PLANT AMELIORATION

Many modifications were performed on the pilot plant during the first weeks of operation. Two general areas of difficulty were encountered at this time: keeping the overall system in a state of dynamic equilibrium, and developing a better method for the return of sludge from the settling tank.

The problem of dynamic equilibrium was solved by installing a sensitive pressure-regulating valve on the influent air line and by taking more care to keep the volumes of liquid in the gas washers constant. The aeration tank was under pressure throughout this period of experimentation, necessitating the maintenance of a constant pressure in the tank.

The sludge return problem was alleviated by replacing the scraper with one of higher speed and installing larger return lines than were used initially.

EXPERIMENTAL PROCEDURE

Making volume measurements, formulating the feed, replenishing the gas washers, and taking samples were performed daily or at two-day intervals throughout the acetate supplement study. The following measurements were made each testing day: volume in the effluent tank, volume remaining in the feed reservoir, volumes of liquid in each bank of gas washers, volume of mixed liquor in the aeration tank, and volume in the settling tank (constant unless a mechanical breakdown occurred). These data were sufficient to calculate loading rates between successive testing times.

Fresh feed was made each testing day to maintain as uniform a feed as possible. Determinations for cyanide and chemical oxygen demand (COD) were performed on the fresh feed daily.

The effluent collected between the testing periods was tested for cyanide, suspended and volatile solids, and COD. The COD was determined on the centrifuged supernatant from the suspended solids determination. The purpose of using the supernatant was to find the percentage of the soluble substrate removed in the treatment process rather than the complete COD of the effluent, since the effluent contained considerable COD exerted by mixed liquor lost in the effluent, an inherent characteristic of extended aeration. The COD determinations were run on the noncentrifuged effluent at intervals to ascertain the total load leaving the plant. In all cases the COD of the centrifuged substrate was 45 to 50% of the COD of the noncentrifuged effluent.

Sodium hydroxide was used in the gas washers to absorb any hydrogen cyanide blown from the system. The 4N base from the washers was collected separately. The second bank of washers was used as a valuation of the efficiency of the first bank. During periods when almost all of the cyanide fed into the system was blown out in the gas, only insignificantly small amounts of cyanide were found in the second bank of washers,

indicating a very high removal efficiency in the first bank. When a nitrogen balance was being executed, boric acid was substituted in the first set of washers to absorb any ammonia in the effluent gases.

The settling tank, which usually contained a significant quantity of sludge, was allowed to drain into the aeration tank by releasing the pressure on the latter. The sample was then taken from the aeration tank. This method assured a representative suspended solids value, as all the solids in the system were in the aeration tank at that time.

The complete nitrogen balance was obtained by analyzing the concentration of nitrites, nitrates, organic nitrogen, and ammonia nitrogen in the feed, mixed liquor, and effluent, and also the gaseous ammonia trapped by the boric acid in the gas washers.

The effluent and gas washer samples were collected between feeding times, and were therefore considered to be composite samples for the period in question. The only possibility of the escape of any component would have been when a gaseous product evolved from the settling tank. Little nitrogen was thought to have been lost in this way because of the relatively short detention time (less than one hour) in the settling tank.

SLUDGE DEVELOPMENT AND ACCLIMATION

Four liters of raw sewage from The Pennsylvania State University sewage treatment plant were put into the aeration tank to develop the activated sludge culture. Tap water was added to bring the volume to the value needed to keep the complete system in equilibrium. The raw sewage was aerated for two days. The unit was then fed the following feed at the rate of 0.22 liter per hour:

2000 mg ammonium acetate per liter
1000 mg monobasic potassium phosphate per liter
Standard BOD dilution water made with distilled water

Building an activated sludge and acclimating it first to the acetate feed and then to cyanide took approximately six weeks. Because mechanical modifications, changes in testing and operational procedures, and changes of the feed solution were made during the acclimation period, the data were fragmentary. For this reason, only minimum, maximum, and median values are given in Table 1, which summarizes the significant data for this term of the experimentation.

The MLSS steadily increased from an initial value of 639 mg/l to about 1400 mg/l at the end of the acclimation period. Rapid growth was expected because of the very high feed COD. The volatile solids did not increase in proportion to the MLSS increase, as expected with the simple soluble organic feed (ammonium acetate). Volatile solids were only 48% of the MLSS at the end of the acclimation period.

The feed solution was applied to the aeration tank at an average rate of 0.22 l/hr during the period. The initial cyanide concentration was 0.32 mg CN^-/l. Removal of cyanide was poor during the first week

TABLE 1. Acetate Acclimation Period

Parameter	Minimum	Maximum	Median
Feed pH	6.1	6.7	6.3
Effluent pH	7.7	8.3	8.1
MLSS, mg/l	556	1466	1017
MLSS, total grams	3.01	10.5	5.19
Volatile solids, %	23.5	70.6	47.7
Feed COD, mg/l	520	1686	1424
COD removal, %*	84.5	97.0	92.0
Cyanide, mg/l	0.32	8.0	3.0

*Based only on soluble COD of the effluent.

of application, but its presence did not interfere with the biological activity of the plant, as exhibited by concurrently high COD removals.

Cyanide concentrations were increased by increments to 8.0 mg/l during the next 20 days. At the time, COD removals were used as the governing parameter for the loading of the system. Removal of cyanide was greater than 80% throughout this period, and COD removals remained above 90%.

Cyanide removal dropped to 5% on the last day, and the cyanide concentration in the feed was immediately lowered to allow the organisms to degrade all the cyanide present and to prevent a lethal concentration of cyanide in the aeration tank.

The fact that cyanide was degraded during the acclimation period showed that the necessary organisms were present to effect treatment. The period of subsequent cyanide increases in the feed solution after the lowering of the cyanide concentration was considered the start of the experimental work.

METHOD OF PRESENTING TEST DATA

The acetate supplement was used as a substrate in the pilot plant for about 14 months following the acclimation period. Testing was not performed continuously, but the unit was kept in operation during periods when testing was suspended.

Growth in the carboy that collected the effluent liquid was thought to be causing an error in some of the data accumulated during the first part of this study. In an attempt to stop any metabolism from taking place after the liquid left the system, formaldehyde was added to the effluent receiving tank each day in concentrations sufficient to kill all organisms in the liquid. It was later discovered that formaldehyde reacts readily with the cyanide ion to form formaldehyde cyanohydrin. Therefore, cyanide in the liquid effluent could not be detected by the silver nitrate titration method used for cyanide determinations. Effluent cyanide values obtained from this point on had little or no significance, but no other data were affected by the error.

The results of the acetate study are reported in two sections: data taken before formaldehyde was added to the effluent carboy (Part 1), and data taken thereafter (Part 2). The former are the more significant, although the latter do contain some information of value to this research.

The data are presented graphically wherever possible. Because of the extended time of the experiment and the excessive amount of data collected, mean values of the measured parameters are the values plotted. The number of individual days of data used to calculate a mean value is indicated by a number in parentheses above the point in question. Mean values of the data for several days tend to average the errors inherent in sampling and analytical testing, thus making the trends of the system more predominant than the daily fluctuations.

RESULTS, PART 1

Cyanide and COD Loadings and Removals. Upon completion of the acclimation period, cyanide was fed to the system at a concentration of 4.0 mg/l. Since cyanide concentrations are inexpressive without loading rates, the cyanide feeding rates were calculated as milligrams of cyanide per gram of mixed-liquor volatile solids (MLVS) per hour.

Figure 2 shows cyanide loading rates and percentages of cyanide and COD removal for 184 days of the study. The acetate supplement was the same as that used during the acclimation period. The COD percentages are calculated for the soluble COD of the effluent rather than the soluble material plus the organisms leaving the system.

Examination of Fig. 2 reveals poor cyanide removal throughout most of the time covered. Three short periods of nearly 100% cyanide removal occurred around days 112, 148, and 165.

The system stopped efficient cyanide degradation on day 115 when a defective pump caused an overload of cyanide to be fed to the activated sludge. The maximum cyanide loading rate accompanied by 100% cyanide degradation was 0.325 mg CN^-/g MLVS/hr. The overloading of cyanide, which had the effect of a shock load, also caused the COD removals to decrease, an indication that the metabolism of all the organisms in the aeration tank was adversely affected.

The cyanide concentration was reduced one-third immediately after the breakdown occurred in order to prevent further deterioration of the activated sludge mass and to allow the organisms to become reacclimated to cyanide. The COD removal efficiency recovered faster than the cyanide removal efficiency, indicating that the cyanide-degrading organisms in the mixed culture were slower-growing than the active mass as a whole.

A second breakdown, on day 152, was also due to a cyanide shock loading. The organisms were metabolizing over 98% of the cyanide fed for about 14 days prior to the shock loading. The cyanide concentrations were increased by approximately 10% per day until day 152, when an increase of 55% was inadvertently applied. An error in the feed make-up caused the shock load to occur at this time. As in the previous breakdown, the COD removal efficiency recovered before the cyanide removal efficiency.

After the breakdown, the cyanide loading rate was reduced to about 0.45 mg CN^-/g MLVS/hr. Good cyanide removals were attained in a few days, and the concentration of cyanide in the feed was again increased incrementally.

Good cyanide removals were recorded, except for one day, until the end of the period covered by Fig. 2. The maximum loading rate achieved during this phase of the work was 0.805 mg CN^-/g MLVS/hr.

During periods of poor cyanide removal the cyanide recovered in the gas washers was always more than double the total recovered in the liquid effluent. Cyanide was never found in the liquid effluent when the gas washers were free of the ion, a fact which indicates that cyanide was blown from the system in the form of hydrogen cyanide gas very readily when the cyanide metabolism was not functioning ideally.

Mixed-Liquor Solids Characteristics. The mixed-liquor solids changed radically throughout the period under discussion. Some of these changes were brought about externally by adding more solids to the liquor; others occurred internally as a function of the metabolism of the organisms in the aeration tank.

Figure 3 shows the solids data for the period. The volatile portion of the sludge remained approximately constant, but the MLSS steadily increased during the first 60 days. The sludge was withdrawn from the tank on day 62, and the heavier inert material, which settled more rapidly than the rest of the sludge, was removed and discarded. Additional activated sludge, which had been acclimated to acetate and cyanide in a separate batch-fed system, was added to the aeration tank to try to obtain a higher active mass in the tank.

The feed solution was also changed within two days of the addition of the new activated sludge. The COD of the feed was left at about 1700 mg/l, but acetic acid was substituted for one-third of the ammonium acetate to reduce the ammonium concentration and to leave the acetate concentration constant. This change lowered the feed pH from about 7.0 to 4.0 and the mixed liquor pH from 8.2 to 7.5.

Figure 3 shows an immediate increase in the volatile solids after this time, with an accompanying increase in the percentage of volatile solids. Although a definite reason cannot be advanced, it is thought that the volatile solids were increased by the new activated sludge from the batch system.

The accompanying increase in the percentage of volatile solids is difficult to explain with the available data. An insoluble nondegradable substance, like a carbonate, was probably present in the system and was dissolved slowly by the increased acidity in the aeration tank after the feed change.

The MLSS reached a plateau of about 55 g on day 70, then dropped to 20 to 25 g and remained there throughout the rest of this part of the study. The drop in solids was definitely a function of the metabolism, since no appreciable loss of solids could be traced to the equipment.

The liquor in the settling tank was cloudy until about day 150, when it became very clear, indicating that the lost solids had left the system in the effluent. The reduction of suspended solids in the effluent was further evidence that less material was being lost by this route after day 150.

The volatile solids reached 90% when the MLSS dropped to 20 to 25 g, and also remained at this level for the duration of the experiment.

Nitrogen Characteristics. An attempt to balance the influent and effluent nitrogen was started on day 137. The percentages found in the balance are presented in Table 2. The value for mixed-liquor nitrogen includes all forms of nitrogen found in the mixed liquor at the time of sampling. If the total nitrogen in the mixed liquor increased between testing periods, the difference between the two values was considered to be nitrogen recovered. Conversely, if this value decreased between testing days, the nitrogen lost was considered to be nitrogen fed to the system, as it ultimately had to be found in the effluent. Samples of the mixed liquor taken from nitrogen and solids testing were considered part of the recovery, and are so entered in Table 2.

The data in Table 2 show that more mixed-liquor nitrogen was lost from the system than was gained, an indication of a net loss of activated sludge during the period. The solids data in Fig. 3 clearly show this to be the case.

TABLE 2. Acetate Study - Summary of Nitrogen Balances, Days 137 to 184

Nitrogen Component	Percent*
Feed	
NH_3-N	75.8
CN-N	17.6
Mixed liquor - N	6.6
Total	100.0
Recovery	
Gas NH_3-N	4.8
Effluent NH_3-N	37.7
Effluent org.-N	25.4
Effluent and gas CN-N	3.8
Effluent NO_2-N	1.8
Effluent NO_3-N	0.1
Mixed liquor samples	3.7
Mixed liquor - N	2.6
Total	79.9

*Based on 22 separate test days.

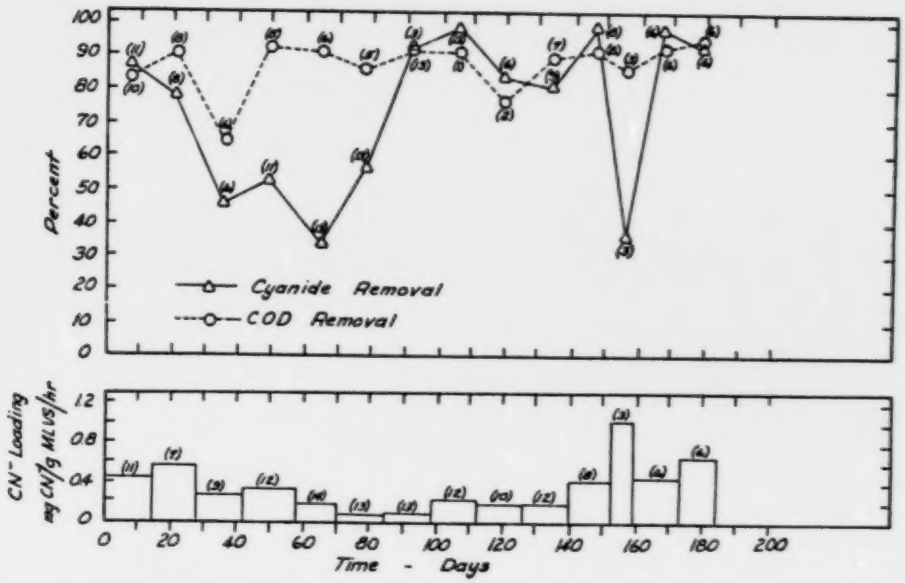


FIG. 2. Acetate study - cyanide loadings and percentages of COD and CN^- removal.

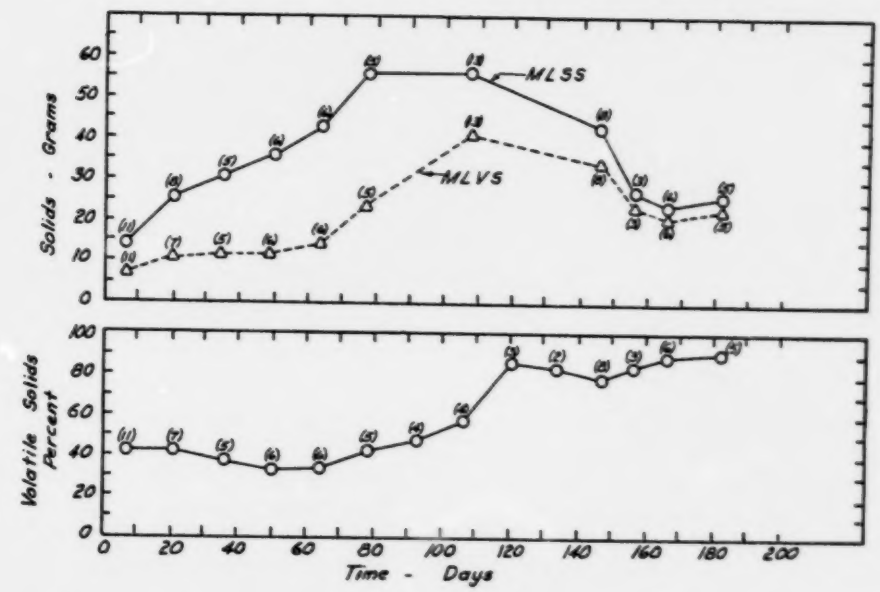


FIG. 3. Acetate study - suspended and volatile solids.

The 20% of the nitrogen fed to the system but not recovered was possibly converted to elemental nitrogen in the settling tank, and therefore could not be determined. A thick sludge layer built up on the bottom of the settling tank throughout most of the testing period. When the blanket was present, particles of agglomerated sludge about 0.5 cm in diameter often floated to the top of the tank. Since the lower portions of this tank were definitely anaerobic, denitrification was probably occurring, and the elemental nitrogen gas formed in the process may have been trapped in the sludge, causing the particles to float. The failure of the feed nitrogen to be oxidized indicated either the absence of nitrifying bacteria or the lack of a suitable substrate or trace element in the feed.

The information obtained from the first part of the acetate study was inconclusive regarding the efficiency of this method of treating cyanide, even though biological degradation of the cyanide was demonstrated.

RESULTS, PART 2

Since cyanide concentrations in the effluent could not be determined accurately because of the formaldehyde in the receiving carboy, the remaining work performed in the acetate supplement study must be considered inconclusive. Cyanide appeared in the exit gases throughout the rest of the experiment except during one short interval. Part 1 of the results supported the data of Nesbitt (29) in showing that when cyanide appeared in the gas it also appeared in the liquid effluent, but the quantities in the liquid were not predictable from the gaseous values alone. After the formaldehyde interference was found, the data accumulated to that time had to be revaluated. Since the test for gaseous cyanide leaving the system was valid, it had to be assumed that cyanide was present in the liquid effluent if it was present in the gases.

Although cyanide was present in the gaseous effluent until this part of the experimentation was nearly complete, COD removals were always greater than 90% but cyanide removals rarely exceeded apparent values of 60 to 70%. This indicated that the system became tolerant of the cyanide ion even though good treatment was not achieved.

The MLSS remained in equilibrium, varying from 17 to 25 g during the remaining 200 days the system was operated. Most of the fluctuations were due to mechanical difficulties rather than the metabolism of the culture in the tank.

The system effectively treated cyanides at concentrations averaging 30 mg CN^-/l with 100% removal for a short time. The average loading rate during this two-week period was 0.2 mg CN^-/g MLVS/hr. Although cyanide was being degraded effectively, the unit was found to be very sensitive to increases in loading rate, a 10 to 15% increase completely upsetting the system for brief periods. Further studies using ammonium acetate as a supplement were therefore discontinued.

DISCUSSION

With extended aeration the complete-mixing activated sludge plant removed COD from an ammonium acetate-cyanide feed, but cyanide removals were generally low and very erratic. The highest cyanide loading rate attained with complete removal was 0.61 mg CN^-/g MLVS/hr. This rate could not be maintained for an extended time, and was considerably lower than that reported by Nesbitt (29) and Ludzack (19).

The mere fact that cyanide was degraded was sufficient evidence that a culture had been developed which was metabolizing the cyanide. The high sensitivity to small cyanide increases and the low cyanide loadings were both considered to be negative characteristics for a treatment method when analyzed in terms of engineering economics. Although this phase of the study yielded a negative result, some positive conclusions were inferred from the research.

The extreme sensitivity of the system to cyanide was apparent throughout the study. Aaslestad (1), using an Arthrobacter culture developed from the activated sludge of this system, found that the presence of ammonia significantly reduced cyanide metabolism in growing cells. Since activated sludge consists of growing organisms, the presence of ammonia is thought to have contributed to the sensitivity experienced when ammonium acetate was used as a supplemental feed. The normal concentration of NH_3-N in the pilot plant study was about 80 mg/l.

Comparison of the recovery rates of COD and cyanide removals after a breakdown indicated that the whole active mass had more resilience than the part responsible for cyanide degradation. COD removal recovered faster than cyanide removal. If the organisms responsible for cyanide degradation grew at a slower rate than the active mass, this phenomenon would be explained. Most of the previous research on cyanide metabolism has demonstrated that the responsible organisms are slow growing (29,32,38).

During periods of poor cyanide removal large quantities of hydrogen cyanide gas were blown from the system. The converse was also exhibited: no cyanide was blown from the system during efficient cyanide removal. The inference from this observation is that the cyanide ion is immediately hydrolized, probably by an extracellular enzyme, during efficient cyanide removal. The biological reaction had to be immediate, or significant quantities of hydrogen cyanide would have been blown from the system or detected in the effluent.

Protozoa and rotifers were present in the mixed liquor, supporting the theory that the biological uptake of cyanide was extremely fast. These organisms always died when treatment efficiency was poor. If free cyanide remained in the aeration tank, they could not exist because of their relatively low tolerance of the ion.

Further attempts to work with the acetate supplemented feed were abandoned because of the sensitivity of the system to cyanide. The supplement contained all the necessary inorganic trace elements needed for bacterial growth, but trace organic compounds, such as vitamins, were

completely lacking. Ludzack* believed that the trace organics may be as essential in an activated sludge plant as the trace inorganics. It therefore seemed necessary to determine whether a supplement containing the trace organics would cause the system to operate more reliably and effectively.

6. SYNTHETIC SEWAGE SUPPLEMENT STUDY

Since the absence of organic trace elements could have been responsible for the unsatisfactory cyanide treatment obtained with ammonium acetate as the supplement in the complete-mixing activated sludge unit, raw domestic sewage would have been the ideal supplementary feed for the next part of the study. It contains all the trace organics necessary for survival of the lower forms of life. But domestic sewage was unsuitable for these experiments because of its variability and because of transportation difficulties. A close simulation was provided by synthetic sewage prepared from a fish food by a method which will later be described.

To determine the possible influence of the age of cells on their ability to metabolize cyanide, both extended-aeration and sludge-wasting studies were conducted with the synthetic sewage supplement.

THE ECONOMIC FACTOR

Economy was a further reason for investigating sewage as a supplemental feed. Biological treatment of cyanide would have little advantage over chemical treatment if its efficiency depended upon the addition of expensive organic nutrients. Most large industrial plants have a considerable domestic sewage flow, which would be the most economical source of the necessary organic load. If a plant producing cyanide waste did not have enough domestic sewage to feed a treatment unit, an agreement with the local sewage authority to tap one of its lines would not be out of the question. Such an arrangement should still be less costly than continuous feeding of a prepared nutrient.

Proof was needed, however, that a complete-mixing activated sludge system with a sewage supplement could achieve the efficiency required for the treatment of industrial cyanide wastes.

PREPARATION OF FEED MIXTURE

The synthetic sewage used for both the extended-aeration and the sludge-wasting study was prepared from a fish food, packaged in granular form, having the following analysis: not less than 40% crude protein, 5% crude fat, 5% crude fiber, and 36% nitrogen-free extract. A quantity of dry food, usually 30 g, was mixed with 500 ml of tap water and homogenized in a blender operated at maximum speed for 5 min. The solution was then poured into a 500 ml graduate and allowed to settle for 15 min. The heavy particles formed a blanket on the bottom, and the

*F. J. Ludzack, personal communication, 1961.

particles lighter than water rose to the surface. It was desirable to separate these two fractions to prevent their separating in the feed carboy and producing a nonhomogeneous solution.

At the end of the settling period, 300 ml of the material remaining in the central section of the graduate was siphoned into another receptacle and transferred to the feed carboy. To make up 20 liters of feed, 80 ml of 1N sodium hydroxide, cyanide stock solution, and tap water were added to the carboy. The sodium hydroxide prevented hydrogen cyanide gas from evolving from the feed mixture.

This method yielded feed mixtures that were very reproducible. The amount of fish food was determined in such a way that the COD of the finished feed would be in the range of domestic sewage (300 to 400 mg/1) without the contribution of the cyanide, which exerts a COD of its own.

EXTENDED-AERATION STUDY

Results of the extended-aeration study are reported for three distinct phases. The first phase, which encompassed about 200 days of operation, ended in a serious mechanical breakdown. The second began with a controlled acclimation period and continued for about 75 days. The system did not break down at that time, but other experimental work using the cyanide-acclimated activated sludge prevented completion of the tests, though the pilot plant remained in operation. The third phase covered the 42 days necessary to determine the ultimate capacity of the system for cyanide.

The solids age throughout the extended-aeration experiments varied from 25 to 40 days.

RESULTS, PHASE 1

Figure 4 shows cyanide loadings, MLSS, and two nitrogen balances obtained during the first 200 days the pilot plant was operated with the synthetic sewage supplement. Cyanide loadings and solids data were taken continuously, and the nitrogen balances were performed over two short periods.

The COD applied to the system remained quite constant at about 350 to 375 mg/1 throughout this phase of the work. Most of the differences in COD during the experiment could be attributed to changes of the cyanide concentration. The COD removals were less than those of the acetate study. The higher effluent COD can be explained by two factors. First, most of the applied COD was in a colloidal state and part of it may not have been degradable, whereas the acetate feed was soluble and if any of it was biologically degradable, all of it had to be. Second, experience in this laboratory has shown that a residual COD is always found, regardless of the degree of treatment. The feed COD of the acetate study was four times greater than that of the synthetic sewage study, but the effluent COD values were only slightly different. This being the case, COD removal efficiency with the sewage supplement would be considerably lower because of the loadings employed.

The synthetic sewage supplemented system removed all cyanide fed to it except during the biological breakdown on day 140 and the mechanical breakdown on day 198. No cyanide removal efficiencies are shown because of the 99+% treatment at all other times.

The MLSS weight in the system dropped sharply from 30 to 10 g during the first 15 days of the experiment. The loss in weight was caused by the abrupt reduction in available food when the system was taken off the acetate and put on the synthetic sewage.

Cyanide was not fed until day 19, as shown in Fig. 4. The loading was raised from 0 to 1.65 mg CN^-/g MLVS/hr in 20 days, and was then allowed to level off for a considerable time. A rise in solids level paralleled the increase of the cyanide concentration. If this rise could be attributed to growth in the system caused by the metabolism of cyanide, it would indicate that cyanide can be a source for synthesis as well as an energy source. In previous work by Nesbitt (29) and Aaslestad (1) it appeared that cyanide could be used only as an energy source.

A sharp peak in cyanide loading can be seen on day 87 in Fig. 4, when an unintended shock load fed the system about 180% more cyanide than it had received previously. This had no detectable adverse effects on treatment efficiency. A similar shock occurred on day 178, again inadvertently, when twice the normal cyanide load was fed the system with no adverse effects. These two shock loads proved that the sewage supplemented system was much less susceptible to a cyanide overdose than the acetate system.

A breakdown occurred on day 140, at which time cyanide treatment ceased. Since all mechanical equipment was operating properly, the breakdown had to be due to the biological process only.

McKinney (25), McCarty and Brodersen (22), and Kountz and Forney (15) have all demonstrated a build-up of biologically inert volatile organic matter in extended-aeration systems with no intentional sludge wasting. This material was thought to be a polysaccharide originating in the cell protoplasm and not degraded during endogenous respiration. With a constant food supply, solids will increase in an aeration tank until the capacity of the system to settle and retain them is exceeded, at which time the excess sludge will be discharged in the effluent. The active mass of an activated sludge operating on extended-aeration principles has been shown to be 10 to 20% of the volatile solids. When a unit starts to release sludge because of the inert build-up, the active mass remaining in the mixed liquor can soon become very small.

It is probable that the biological breakdown on day 140 occurred because the system had started discharging solids, thereby reducing the active mass in the aeration tank and overloading the remaining active mass with cyanide.

Figure 5 shows the effluent suspended solids in the system for a period of two weeks before the breakdown, through the failure and reacclimation periods, to the point at which cyanide was again being

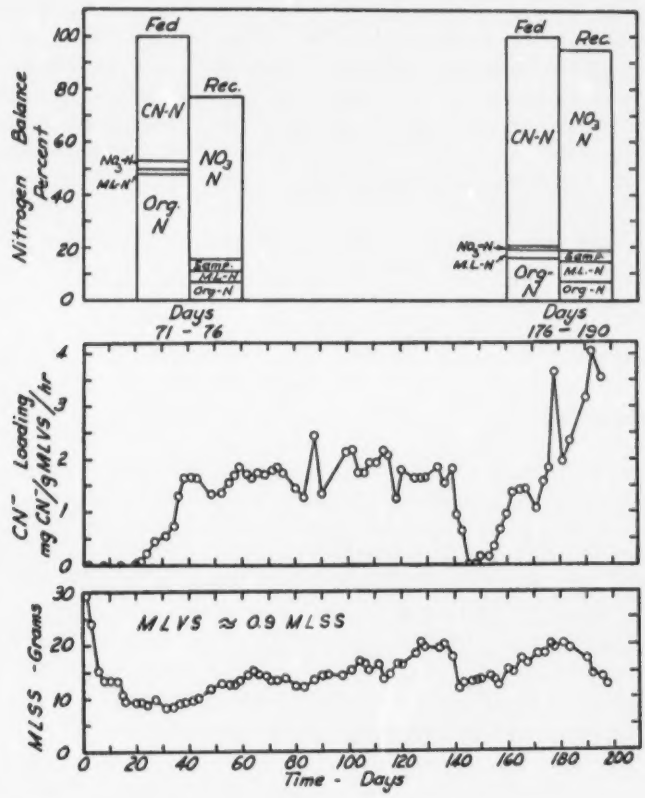


FIG. 4. Synthetic sewage study, Phase 1 - solids, CN^- loadings, and nitrogen balances.

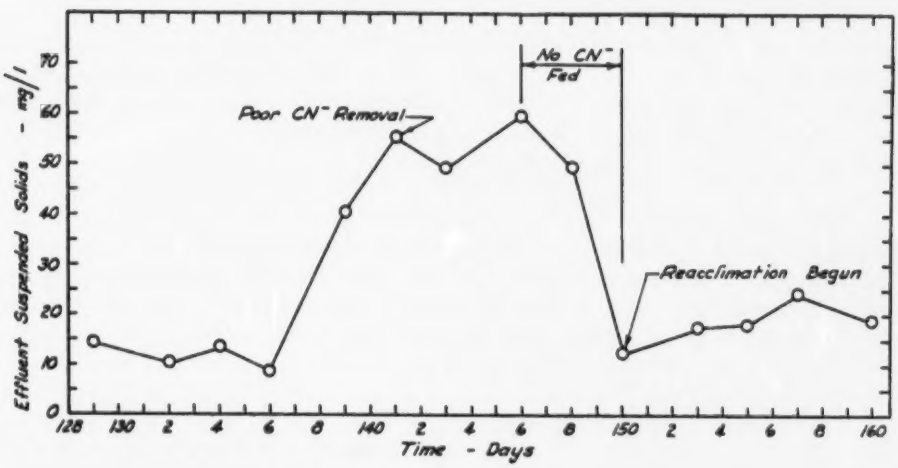


FIG. 5. Synthetic sewage study, Phase 1 - effluent suspended solids, days 129 to 160.

completely removed biologically. An effluent suspended solids level of 8 to 20 mg/1 was the value normally found throughout this phase of the experimentation. The effluent solids rose to 40 mg/1 on day 139. The cyanide loading rate was not reduced, however, because the system had gone through many cycles of losing solids without a biological breakdown. But the unloading of solids was much more severe than had been noticed previously, and a complete biological breakdown occurred between days 139 and 140, the unit removing only 35% of the applied cyanide.

The cyanide concentration in the feed was reduced to two-thirds, but cyanide removal remained poor. Only the synthetic sewage, with no cyanide, was fed between days 145 and 150. The effluent suspended solids returned to a normal value on day 150, at which time cyanide was again applied to the system. One hundred percent cyanide removal occurred immediately, and there were no further biological breakdowns during this phase of the research.

On day 180 the MLSS weight in the system reached 20 g, the same value reached on day 138 before the biological breakdown. Again the solids in the system decreased appreciably, as can be seen in Fig. 4. Effluent suspended solids also increased, about like those shown in Fig. 5, but cyanide removals remained at the 100% value. The only difference in operation between the two periods was that at day 140 the cyanide concentration in the feed was being held constant, whereas it was being increased daily during the end of this phase. The first period could therefore be considered to be in equilibrium; the second was probably in a state of more active growth.

It should be noted that the increase in cyanide loadings after day 180 was caused by two factors: (a) the cyanide concentration in the feed was being increased, and (b) the volatile solids in the system were concurrently being reduced, as seen from the solids curve in Fig. 4. The combination of the two factors had a more severe effect on the loading rates than the increase in cyanide concentration alone.

Nitrogen balances performed during this phase of the research are also shown in Fig. 4. The two sets of histograms represent periods when moderate and high cyanide loadings were applied to the system. The same procedure was used for this balance as was used for the balance in the acetate supplemented study.

The most significant information in these data is the high nitrate recovery and the lack of ammonia in the effluent. The nitrates nearly equaled the cyanide nitrogen fed to the system, indicating that the cyanide nitrogen is about quantitatively converted to nitrates. Ludzack (19), using a sewage supplemented pilot plant, reported ammonia in the effluent at all times, even though the feed contained none.

This phase of the study ended abruptly when a pressure increase in the aeration tank forced two-thirds of the solids out of the system. After this failure the aeration tank was operated under atmospheric pressure, and the effluent gas sampling was performed by a partial vacuum.

RESULTS, PHASE 2

Phase 2 was started shortly after the mechanical breakdown that ended Phase 1. The aim in this phase was to allow the system to reach a state of equilibrium with only the synthetic sewage and then start it on cyanide, so that any fundamental differences in the measured parameters could be observed.

Figure 6 shows the MLSS results collected over the duration of this experiment. The solids in the aeration tank at the outset totaled 4 g, or less than 1000 mg/1. After 20 days the solids had grown to about 7 g and appeared to be in a state of equilibrium. The evidence of equilibrium displayed by the leveling off of the solids curve between days 15 and 25 was checked by determining the food/microorganism (F/M) ratio, as described by McKinney (25). For an activated sludge system to operate in equilibrium with only sufficient food being applied to keep the cells alive without autodigestion, the F/M ratio should equal 0.45. At day 15, the organic loading on the plant was about 300 mg BOD/1/day, with about 9 liters applied per day, giving a food loading of 2700 mg per day. This loading was applied to about 7000 mg of MLSS, of which about 6300 mg were volatile. Therefore, the F/M ratio was 2700/6300, or 0.43, a value in close agreement with the 0.45 value cited by McKinney for equilibrium conditions.

The nitrogen data collected over this period (Fig. 7) also confirmed that the unit was stabilized. The organic and nitrate nitrogens recovered in the effluent reached equilibrium shortly after day 15, as did the nitrate nitrogen in the mixed liquor (not shown in Fig. 7).

Cyanide was applied for the first time on day 25. The loadings were kept low during the next week, after which they were steadily increased, as shown in Fig. 8. A mechanical breakdown occurred on day 48, requiring reacclimation. Nitrogen data were not continued past the breakdown, but almost all other data were taken until day 71. After day 71 the system was kept in operation, but data collection was discontinued to allow more time for work on the radioactive study discussed in Section 7. Phase 3 of this experiment, concerned with the loading of the unit to its ultimate capacity, was actually an extension of Phase 2, as no intermittent breakdowns occurred.

The solids in the system increased as cyanide loadings were increased. Whether this was due to the presence of new active cells or to a polysaccharide build-up is uncertain, but the fact that the increase came after the cyanide addition indicates the probability that the cyanide carbon was used for synthesis of new protoplasm.

Although sludge wasting, as such, was not practiced during this phase of the experimental work, some sludge was withdrawn for sampling and for unrelated biochemical studies. Three percent of the sludge, and often as much as 6%, was withdrawn every second day for testing.

Washington and Symons (48), trying to determine the polysaccharide accumulation in extended-aeration systems, concluded that at a loading of 40 lb COD/day/1000 cu ft total aeration capacity, volatile sludge

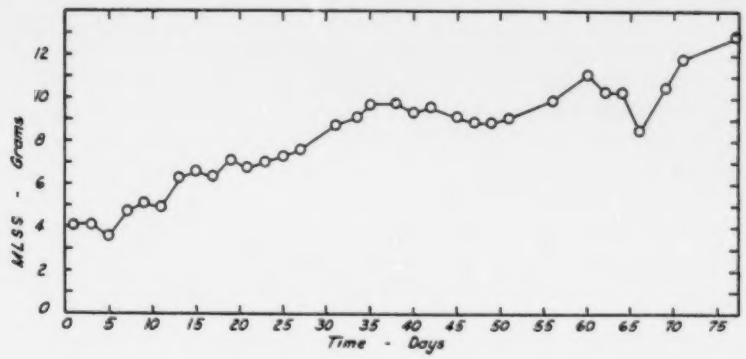


FIG. 6. Synthetic sewage study, Phase 2 - mixed-liquor suspended solids.

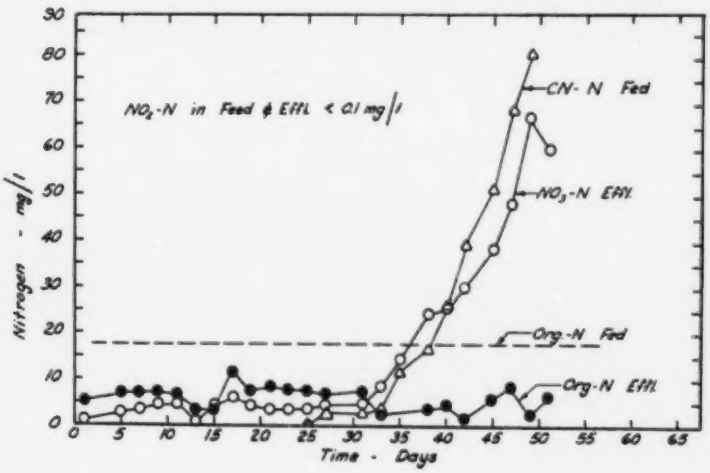


FIG. 7. Synthetic sewage study, Phase 2 - nitrogen characteristics.

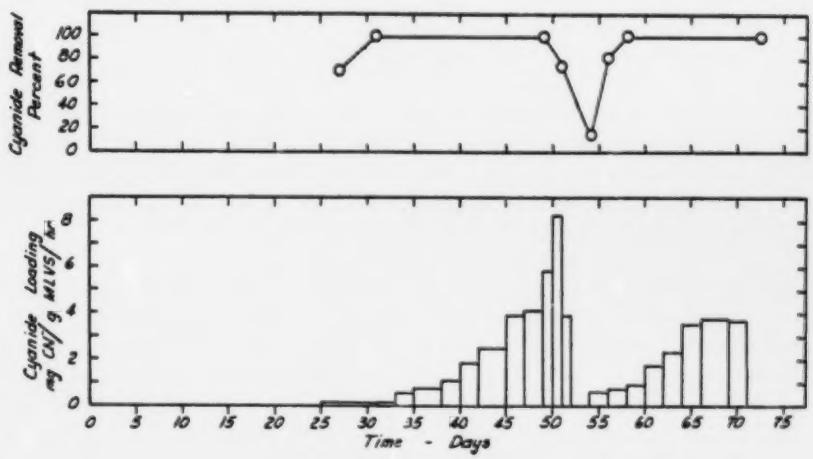


FIG. 8. Synthetic sewage study, Phase 2 - cyanide loadings and removals.

accumulates at the rate of about 10 to 15% of the COD of the substrate removed. As shown in Fig. 6, the sludge increased from 7000 mg to 12,500 mg between days 25 and 75. The average COD loading was about 2250 mg/day, with 80% removal. Therefore, 180 to 270 mg/day of inert organic material should have been accumulating in the system under discussion. Sampling procedures necessitated the removal of 150 ml of sludge every other day, with an average weight of 140 mg/day over the period. According to Washington and Symons, the new increase in volatile solids should therefore have been in the range of 40 to 130 mg/day or 2000 to 6500 mg for the 50 days. Assuming 90% volatile solids in the sludge, the actual increase was from 6300 to 11,200 mg, or 4900 mg, a value midway in the prescribed range.

Research to be discussed in Section 7 shows that a portion of the cyanide carbon was used to produce new cell material. Approximately 7.9 g of cyanide carbon were fed to the system over this 50-day period. Assuming the average composition of cells (15) to be $C_5H_7O_2N$, this carbon would produce about 9.7 g of cells if it is further assumed that one-third of the carbon was used for respiration and two-thirds for cell synthesis.

Although the polysaccharide accumulation appeared to account for the solids increase during this experiment, cell synthesis using the cyanide carbon could also account for the increase plus some extra that possibly was lost in the effluent. Therefore, no definite conclusion could yet be drawn as to the cause of the solids increase in the aeration tank during this phase of the experimentation.

The nitrogen data up to and including the breakdown on day 48 have a great deal of significance. The synthetic sewage contained 17.5 mg/l organic nitrogen, expressed as N, and negligible concentrations of all other nitrogen forms. This feed was held constant throughout Phase 2, only the cyanide concentrations being altered. The organisms therefore had to obtain all their nitrogen from the sewage until cyanide was added to the feed on day 25. The cyanide added, expressed as cyanide nitrogen concentration, is shown in Fig. 7. As the cyanide concentration was raised, the nitrate concentration in the effluent nearly paralleled it, which indicated that the biota in the system almost quantitatively converted the cyanide nitrogen to nitrates.

Ammonia and nitrite nitrogen were absent from the mixed liquor and effluent. Organic nitrogen was found in the effluent, as would be expected because of the constant discharge of some suspended solids. Figure 7 shows the organic nitrogen to be quite low and fairly consistent throughout the period.

When the system failed because of an overload of cyanide on day 48, the nitrification was immediately reduced, although not completely stopped.

RESULTS, PHASE 3

The pilot plant was operated as a culture source for other experiments during the next 60 days. No attempt was made to perform analytical

tests during this period, but the unit was checked daily for cyanide removal efficiencies and no cyanide left the unit untreated. The resumption of testing was considered to be day 1 of Phase 3.

Figure 9 shows the MLSS and the cyanide loadings rates for Phase 3. The total weight of mixed liquor was about 17.5 g on day 1, or an increase of about 4.5 g during the 60 days the unit was not tested. Since the cyanide loading rate was decreased from the end of Phase 2 to the start of Phase 3, the increase in solids can be attributed to polysaccharides only.

The solids increased quite sharply when the cyanide loading rate was increased. Between day 4 and day 41 they went up from 17.5 g to 30.2 g, a net gain of 12.7 g. Because the cyanide carbon was thought to be used for cell synthesis, most of the increase in cell weight was believed to be due to the cyanide loading rate.

The total cyanide fed during the period was 50 g, of which 23.1 g were carbon. If 15.4 g of this carbon, or two-thirds of the total, was used for synthesis, the total cells produced would be 29.4 g, assuming the composition of the cells to be $C_5H_7O_2N$. A balance of the solids during this period showed 3.3 g of solids lost in the effluent and 12.9 g removed for sampling, or a total of 16.2 g wasted from the system. Adding the 16.2 g wasted to the 12.7 g of new sludge accumulated in the aeration tank, the total new cellular material was 28.9 g, or 98.5% of the theoretical value of 29.4 g. That two-thirds of the cyanide carbon had been used for cell synthesis therefore appeared to be a fact.

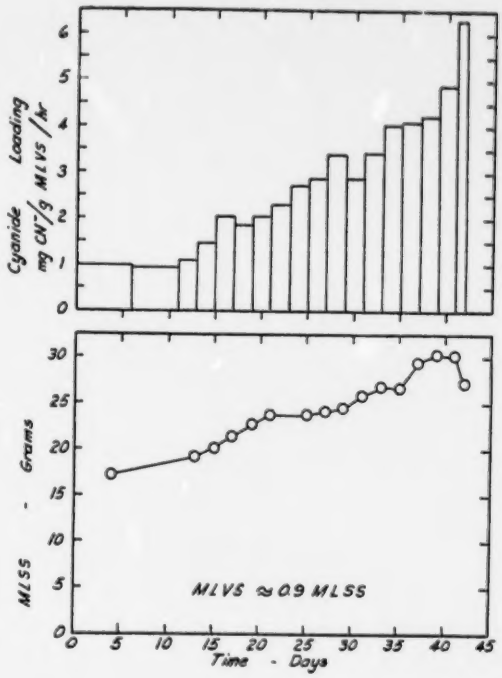
The cyanide loading rates, also plotted in Fig. 9, show little increase during the first 10 days, but a steady rise after that time. It was very difficult to load the plant by equal increments, because the solids were steadily increasing and the influent rate was decreasing. The maximum loading attained was 4.9 mg CN^-/g MLVS/hr with complete removal of the cyanide. At this loading, the unit was being fed 540 mg CN^-/l at the rate of 0.244 l/hr, creating a detention time of 22.5 hr in the aeration tank.

One of the most significant aspects of Phase 3 was again the nitrogen results. Figure 10 shows the cyanide nitrogen fed and the nitrate nitrogen recovered. In terms of concentration, the two curves nearly paralleled each other until day 39, at which time the effluent nitrate broke away from the feed cyanide, giving the first indication that the system was not operating at equilibrium. The same phenomenon was apparent during the breakdown on day 48 of Phase 2.

A more detailed study of the nitrogen recovery data is shown in Fig. 11, where the concentrations of all forms of nitrogen found in the mixed liquor and the effluent are plotted for only the last part of the results. The significant behavior occurred preceding and during the time when cyanide was not removed effectively. Cyanide first appeared in the effluent and the gas washers on day 42.

The nitrates were consistently higher in the mixed liquor than in the effluent except during the breakdown. Since the cyanide concentration was being steadily increased and the mixed liquor was immediately

FIG. 9. Synthetic sewage study, Phase 3 - solids and cyanide loading rates.



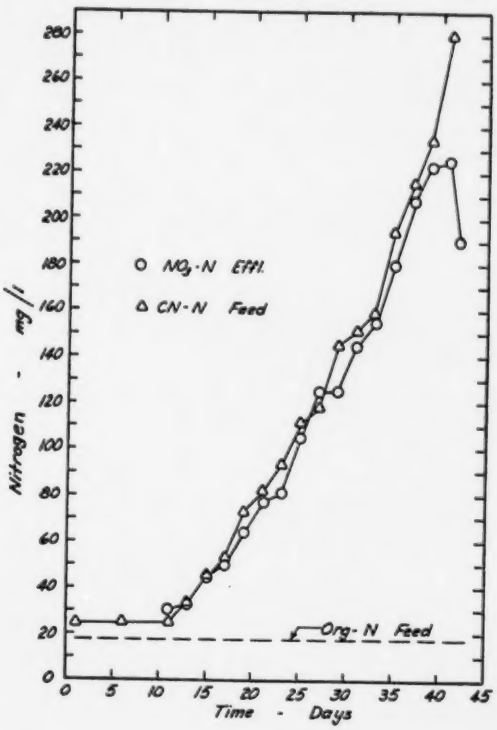


FIG. 10. Synthetic sewage study, Phase 3 - effluent NO_3-N and feed CN-N.

FIG. 11. Synthetic sewage study, Phase 3 - nitrogen data preceding and during biological failure.

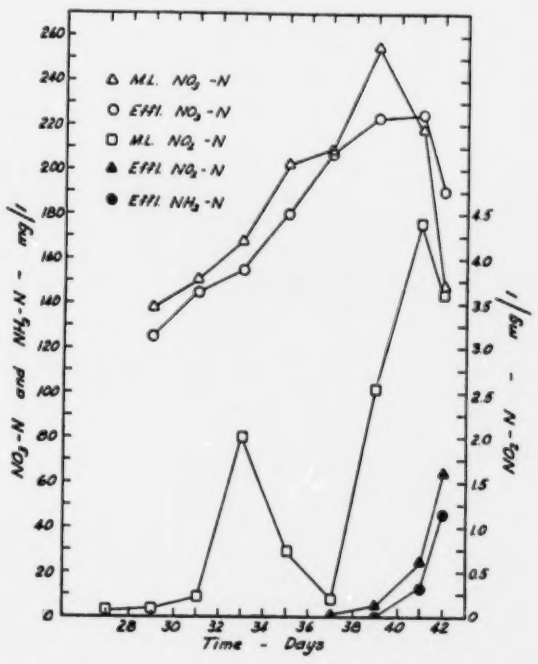


TABLE 3. Extended-Aeration Study - Nitrogen Balances for Phase 3

Day	Feed, mg						Recovery, mg							Percent Recovery
	Org-N	CN-N	NO_3-N	NO_2-N	ML-N	Total	NO_2-N	NO_3-N	NH_3-N	Org-N	Samp-N	ML-N	Total	
11	404	560	12.0	0.2	0.0	976	0.4	749	0.0	336	0.0	0.0	1085	90.0
13	254	485	7.4	0.1	3.0	760	0.2	545	0.0	0.0	77.4	0.0	622	82.0
15	262	679	10.0	0.2	0.0	951	0.2	732	0.0	0.0	58.9	13.0	804	84.3
17	235	683	8.2	0.1	0.0	926	0.1	735	0.0	33.0	59.5	301	1128	122
19	236	979	6.7	0.3	147	1369	0.1	981	0.0	19.1	67.7	0.0	1068	78.5
21	236	1110	30.2	0.3	0.0	1377	0.2	1210	0.0	0.0	62.6	608	1881	137
23	219	1170	9.3	0.1	146	1544	0.2	1185	0.0	0.0	79.5	0.0	1265	82.0
25	232	1481	9.3	0.1	0.0	1722	0.2	1540	0.0	0.0	80.0	242	1875	109
27	262	1760	7.9	0.3	0.0	2030	0.2	2060	0.0	0.0	83.5	121	2265	115.5
29	222	1835	7.7	0.3	0.0	2065	0.1	1765	0.0	0.0	89.8	129	1974	95.6
31	220	1900	1.0	0.3	0.0	2121	0.1	2020	0.0	25.0	91.5	60.0	2197	103
33	234	2262	8.4	0.3	0.0	2495	0.1	2240	0.0	28.4	93.4	287	2649	102
35	219	2440	10.9	0.3	0.0	2670	0.2	2650	0.0	16.6	88.4	1.0	2756	103
37	222	2820	9.3	0.3	0.0	3052	0.3	2910	0.0	0.0	98.0	387	3395	109.5
39	198	2740	8.3	0.2	0.0	2947	1.4	2910	0.0	0.0	101	394	3406	115
41	203	3380	7.8	0.3	104	3695	7.7	2900	66.7	0.0	115	0.0	3088	84.0
42	98	2070	3.2	0.2	16.0	2188	12.2	1430	340	60.0	111	0.0	2064	94.5
					Total	32888						Total	33522	

Recovery over period = $\frac{33522}{32888}$ = 101.8%

exposed to the increase, whereas the effluent tank was a composite of two days' operation, the greater nitrate concentration in the mixed liquor appears to be reasonable.

Biological degradation of any substance, as well as further growth, was prevented in the effluent-receiving carboy by adding concentrated sulfuric acid to the effluent to give a concentration of 1500 mg H_2SO_4/l, as specified in Standard Methods for the Examination of Water and Wastewater (3) for the storage of samples to be used for nitrogen balances.

Nitrites were always present in the mixed liquor, in concentrations usually less than 0.5 mg/l except for an increase on day 33, when they rose to 2.0 mg/l. (The last figure may be high because of analytical errors.) The nitrites in the effluent were less than 0.01 mg/l throughout the same period. Day 39 saw a marked rise of the nitrite values in both the mixed liquor and the effluent. This rise continued until biological breakdown occurred when an excess cyanide loading was added on day 41. Nitrification to nitrates then dropped sharply, as did nitrite formation in the mixed liquor, but the effluent nitrite and ammonia concentrations continued to increase.

The nitrogen data served as a warning of impending biological breakdown. Hence the discontinuation of cyanide metabolism may not have been dependent upon the cyanide, but rather upon the increase of nitrite in the mixed liquor, which stopped the nitrogen cycle at an intermediate point.

The solids exhibited a decrease in flocculation ability at the time of the breakdown, as shown by the loss of solids on day 42 in Fig. 9.

Nitrogen balances performed continuously during this phase of the work are summarized in Table 3. The overall nitrogen recovery for the period was 101.8%. This table again shows the correlation between cyanide nitrogen fed and nitrate nitrogen recovered. All data were for a period of two days between tests, except that day 42 was a one-day period because the biological breakdown, was apparent.

CONCLUSIONS

From the results of Phase 3 it must be concluded that the organisms present utilize cyanide carbon for synthesis as well as for respiration, and that ultimately the cyanide nitrogen is converted quantitatively to nitrate. Complete nitrification ceases as the cyanide loading rate approaches its maximum limit of about 5.0 mg CN^-/g MLVS/hr, a fact that could be significant in the operation of a prototype plant.

SLUDGE-WASTING STUDY

Since all of the preceding experiments employed the extended-aeration principle, the cell population was relatively old (25 to 40 days). To compare the cyanide-metabolizing ability of younger cells, a sludge-wasting study was performed immediately after the failure in Phase 3 of the extended-aeration study. The average age of cells in this experiment was 11 days. Sludge wasting causes the biota to grow at a more rapid

rate, because fewer organisms are present to metabolize the same amount of substrate.

WASTING PROCEDURE AND FEED

Ideally, the amount of sludge wasted should be equivalent to the growth rate, so that the solids concentration in the aeration tank remains constant. The wasting procedure can be done continuously or on a batch basis. Symons (43) reported that in bench-scale activated sludge units the method of sludge wasting, batch or continuous, has no effect on the mixed-liquor solids concentration. Batch sludge wasting was chosen for this study because it required no additional equipment.

The system was fed the same synthetic sewage as that used for the extended-aeration studies, at about the same feed rates. Sludge was wasted from the aeration tank at 48-hr intervals in an attempt to keep the MLSS concentration approximately 2000 mg/l.

CYANIDE LOADINGS

Figure 12 gives the cyanide loading rates throughout this experiment. Two loading rates are indicated in each of the histograms to show the difference caused by the wasting of sludge. Since appreciable growth took place between the times when the sludge was wasted, the loading rates were changed proportionately during the same period. The maximum rate therefore occurred immediately after wasting, when the solids were at a minimum; the minimum rate occurred when the solids were at a maximum, immediately before the sludge was wasted.

In the early stages of this study, loading rates were increased quite rapidly to determine the possible effects of shock loadings at these levels. Though the loading was more than doubled on day 6, no cyanide was present in the gas or the effluent and no adverse effects were observable in the operation of the system. The comparable cyanide concentrations were 20 mg/l and 49 mg/l between the two periods. On day 8, the next feed day, the concentration was raised to 99 mg CN^-/l with no adverse effects. The ability of this system to absorb sharp increases in loading rates without an upset of the metabolism showed it to be much less sensitive than the acetate supplemented system.

Cyanide first appeared in the exit gases and in the effluent on day 21. Other measured parameters, which will later be discussed, indicated that on day 19 a biological breakdown was approaching. The maximum loading rate was 5.95 mg CN^-/g MLVS/hr, and the minimum for the day was 4.98 mg. Since the system failed in this region, the maximum cyanide loading that was metabolized completely was in the range of 4.76 to 5.36 mg CN^-/g MLVS/hr.

SOLIDS

A large quantity of solids had to be wasted from the extended-aeration system in order to have a mixed-liquor concentration approximating 2000 mg/l at the start of this study. During the cyanide reacclimation period after the breakdown in Phase 3, the solids concentration remained

undisturbed. Sludge wasting was begun on day 3, as shown by the vertical line at day 3 on the MLSS curve in Fig. 12. From that day on, the solids were wasted at 48-hr intervals (Fig. 12, vertical lines).

The growth rate during the experiment was quite consistent, as demonstrated by the slope of the lines between successive wasting times. Only three days were greatly different. The solids did not appear to increase at a normal rate between days 11 and 13, but this inconsistency was thought to be caused by the use of nonrepresentative sample in the solids determination. Although solids did increase from day 17 to day 21, the rate of increase was less than normal. The cyanide loading limit was being reached at the same time, and was probably inhibiting the growth characteristics of some of the organisms present.

Also given in Fig. 12 is the effluent suspended solids concentration over the period. The last two days show a marked rise, indicating that the metabolism of the organisms in the aeration tank was altered and the flocculation characteristics were changed. The fact that more solids were leaving the system throughout these two days than during most of the rest of the experiment also contributed to the reduction of sludge growth during these days.

Effluent quality, as indicated by the suspended solids in the effluent, improved immediately after sludge wasting began. The increase on day 9 was caused by a loss of solids when the aeration tank effluent line became clogged and then was released, thus overloading the settling tank and discharging some mixed liquor over the effluent weir. This increase in concentration cannot be attributed to the biota as can the increase on the last two days of the experiment.

The average weight of suspended solids in the aeration tank after the initial wasting of day 3 was 11.68 g. This value was calculated by averaging the weight between testing periods. Since the normal volume of the liquor in the aeration tank was 5.2 liters, the average MLSS concentration for the experiment was about 2240 mg/l.

The total weight of sludge wasted during the 18-day period from day 3 to day 21 was 19.13 g, or 1.06 g/day. Therefore, the average age of cells in the sludge-wasting experiment was 11 days.

NITROGEN

The nitrogen concentrations in the feed, mixed liquor, and effluent are presented in Fig. 13. Nitrate nitrogen increased parallel to feed cyanide nitrogen, in a manner similar to that of the extended-aeration experiments. When biological failure occurred, the nitrate concentration in the mixed liquor fell below the feed cyanide nitrogen concentration, and the nitrite concentrations of the effluent and mixed liquor rose as they did during the extended-aeration experiments.

The nitrite concentrations at the beginning of the test period were considerably higher than had previously been observed in this system. Since the unit was reacclimated to cyanide immediately after the extended-aeration experiments, the high nitrite concentration found immediately

before and during a biological failure was probably carried over into this experiment. The final dosage of cyanide in Phase 3 of the extended-aeration work was sufficient to cause a residual cyanide concentration in the aeration tank high enough to be lethal to the majority of the organisms. The nitrite determinations in Fig. 13 indicate that the Nitrobacter were slower growing than the Nitrosomonas, but they were present in sufficient quantities to convert nearly all the nitrite to nitrate by day 15.

Ammonia did not appear in either the mixed liquor or the effluent during the final stages of this experiment as it did in the extended-aeration study. The ammonia fraction might have appeared if a cyanide dose had been applied in sufficient concentration to kill many of the organisms. The failure of this system was detected by the presence of small amounts of cyanide in the effluent, but the biota had not been affected to a great extent, as revealed by microscopic examination of the sludge at the time of the failure. Therefore, most of the nitrifying organisms were probably alive at this time and were able to convert the nitrogen to the oxidized forms.

Table 4 is a compilation of the nitrogen balances performed during this experiment.

CONCLUSIONS

It can be concluded from the sludge-wasting experiment that the metabolism taking place in the young culture is nearly identical with metabolism in the old culture (extended aeration). The ultimate cyanide loading limit was found to be 4.76 to 5.36 mg CN^-/g MLVS/hr, a range which brackets the loading of 5.0 mg CN^-/g MLVS/hr determined in the extended-aeration experiments.

DISCUSSION

CYANIDE DEGRADATION

With the synthetic sewage as supplemental feed, cyanide degradation was much more reliable than with the acetate supplement. The maximum cyanide loading rates were determined to be approximately 5.0 mg CN^-/g MLVS/hr in both extended-aeration and sludge-wasting studies. Ludzack (19) obtained a maximum loading rate of 3.0 mg CN^-/g MLVS/hr with a sewage and dextrose supplement. Nesbitt (29), using cyanide as the only source of carbon and nitrogen, achieved a loading rate of 10.5 mg CN^-/g MLSS/hr. The findings of the present work fall between those values. It should be pointed out, however, that the results of these three studies are not strictly comparable.

Ludzack used a sewage-dextrose supplemental feed in a complete-mixing system. The dextrose may have caused a predominance of organisms other than those that biologically degraded the cyanide ion, and competition for some of the food source could therefore have been present. Moreover, most of the individual experiments performed by Ludzack were of only three weeks duration. Since time plays such an important role

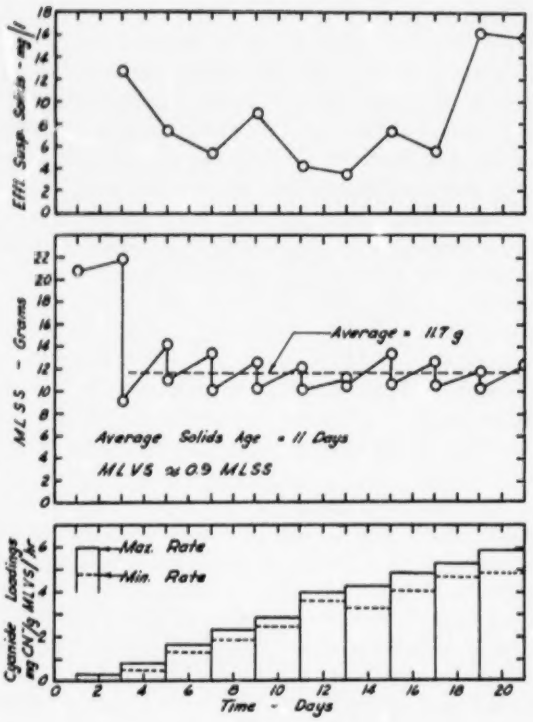


FIG. 12. Sludge-wasting study - cyanide loadings, effluent and mixed-liquor solids.

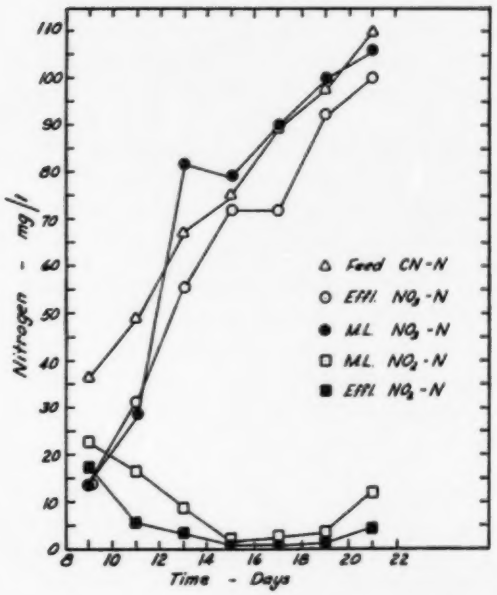


FIG. 13. Sludge-wasting study - nitrogen characteristics.

TABLE 4. Sludge-Wasting Study - Nitrogen Balances

Day	Feed, mg						Recovery, mg					Percent Recovery
	Org-N	CN-N	NO_3-N	NO_2-N	ML-N	Total	NO_2-N	NO_3-N	Samp-N	ML-N	Total	
11	245	685	33.6	0.2	0.0	963.8	90.8	494	262	8	854.8	88.8
13	236	910	12.9	0.5	0.0	1159.4	59.6	862	247	216	1384.6	119.5
15	244	1050	10.6	0.3	0.0	1304.9	15.1	1125	77.5	88.8	1306.4	100.1
17	240	1220	11.1	0.3	0.0	1471.4	13.6	1115	368	121	1617.6	109.5
19	215	1205	10.0	0.3	105.0	1535.3	21.0	1350	304	0.0	1675.0	109.0
21	227	1440	11.7	0.3	0.0	1679	69.0	1490	252	81.0	1892.0	113
					Total	8113.8				Total	8730.4	

Recovery over period = $\frac{8730.4}{8113.8}$ = 107.5%

in any biological system, the longer duration of the experiments here described provides a more reliable base for predicting the behavior of a prototype plant. The importance of the time factor was quite apparent in Phase 1 of the extended-aeration study. The biological failure on day 140 would never have materialized if the observation time had been limited to Ludzack's three weeks. The fact that the system can fail from internal forces only when operating at equilibrium is another very significant consideration in setting up procedures for a prototype plant.

Nesbitt's experimental unit was operated on a principle completely different from that of the present research. Although his feeding was essentially continuous, the rest of his work was carried out on a batch-type basis. In addition, cyanide was the only source of carbon and nitrogen fed the system. The mixed culture normally found in activated sludge plants treating organic substances could not survive on a feed of this kind. Hence the overall metabolism involved in Nesbitt's work has no direct relationship to the results of the present study.

This research showed the synthetic sewage supplemented plant to be much less sensitive to shock cyanide loading than the acetate supplemented unit. The period of exposure to cyanide and the type of supplemental feed are two possible explanations for the difference in characteristic behavior. Since the period of exposure to cyanide in the acetate study was greater than one year, it is unlikely that this factor had any effect. The better nutritional balance of the synthetic sewage feed is believed to have made the culture more stable, especially under the adverse condition of a cyanide shock loading. A third factor, the absence of high concentrations of ammonia in the sewage supplement, may very well account for the difference in performance.

Although both extended-aeration and conventional operations yielded about the same maximum cyanide loading rates, the loading on a prototype plant would be at a lower rate to allow for variable waste quantities and possible mechanical difficulties, and thus to prevent biological breakdown. A loading of about 3.5 mg CN^-/g MLVS/hr is thought to be a safe operating rate.

MIXED-LIQUOR CHARACTERISTICS

The mixed-liquor characteristics were similar to those found in any activated sludge plant. The breakdown of the extended-aeration system on day 140 of Phase 1 is an indication that sludge wasting is beneficial. During that part of the experimentation no sludge was wasted intentionally and a biological failure occurred. It was thought to have been caused by the failure of the mixed liquor to flocculate in the settling tank, owing to a maximum polysaccharide accumulation in the mixed liquor. This failure definitely confirmed that sludge wasting is needed if a unit is to be operated for long periods of time on a constant feed. Until the solids accumulation had reached the critical value, however, the extended-aeration system treated cyanide as effectively as the sludge-wasting system.

Within the range tested, the solids age did not appear to have any effect upon cyanide treatment. The extended-aeration experiment produced solids ages ranging from 25-40 days; in the sludge-wasting experiment

the average solids age was 11 days. All measured characteristics of both systems were nearly the same.

Wasting of solids is thought to be important because it prevents critical polysaccharide accumulations, thereby eliminating the possibility of uncontrollable loss of sludge when the maximum solids concentration is reached. Slug wasting or continuous wasting can be practiced with the same results, provided that the maximum solids value is not reached in the aeration tank.

The sharp rise in solids concentration after the initial dose of cyanide in Phase 3 of the extended-aeration experiment indicated that cyanide could be used as a source of carbon for cell synthesis as well as for respiration. This was most apparent during Phase 3 because of the high concentration of cyanide fed to the system (over 500 mg/l). Since the feed carbon was mainly cyanide carbon, the very fast rate of growth during the experiment can be attributed to cyanide.

NITROGEN CONVERSIONS

The major nitrogen sources in the system were organic nitrogen and cyanide. The organic nitrogen was partially converted to cell material and partially to nitrates, as determined by nitrogen recoveries (Fig. 7) taken when no cyanide was fed the system and equilibrium conditions were being established.

The cyanide nitrogen was quantitatively converted to nitrates during all experiments when the unit was effectively removing cyanide and was not approaching a maximum cyanide loading rate. Immediately before and during a biological failure the nitrate conversion was incomplete, and nitrite and ammonia appeared in the effluent. No ammonia appeared in the effluent during the failure in the sludge-wasting experiment. Since the failure was detected before the cyanide ion concentration in the mixed liquor became high enough to kill the majority of the biota, the organisms that convert ammonia to nitrites were probably unaffected.

The Phase 3 results for extended-aeration are significant because ammonia was present in the effluent immediately before and during the biological failure. Since the system was being fed only 17.5 mg of organic nitrogen per liter, the fact that 45 mg NH_3/l was recovered in the effluent during the last stages of the experiment indicated that the cyanide nitrogen was converted to ammonia, which in turn was converted to nitrites by Nitrosomonas organisms, the nitrites being further oxidized to nitrates by Nitrobacter. The failure of the culture to convert nitrites to nitrates upon the approach of the maximum cyanide loading indicated that the Nitrobacter organisms had reached their limit and were unable to convert all the nitrites to nitrates. As the cyanide concentration neared the maximum, nitrite production was reduced and the ammonia concentration rose. This phenomenon occurred regardless of the type of plant operation being employed at the time.

The Nitrobacter organisms seem to have been slower growing than the Nitrosomonas under the conditions prevailing in these experiments. The high nitrite concentrations in the mixed liquor and effluent at

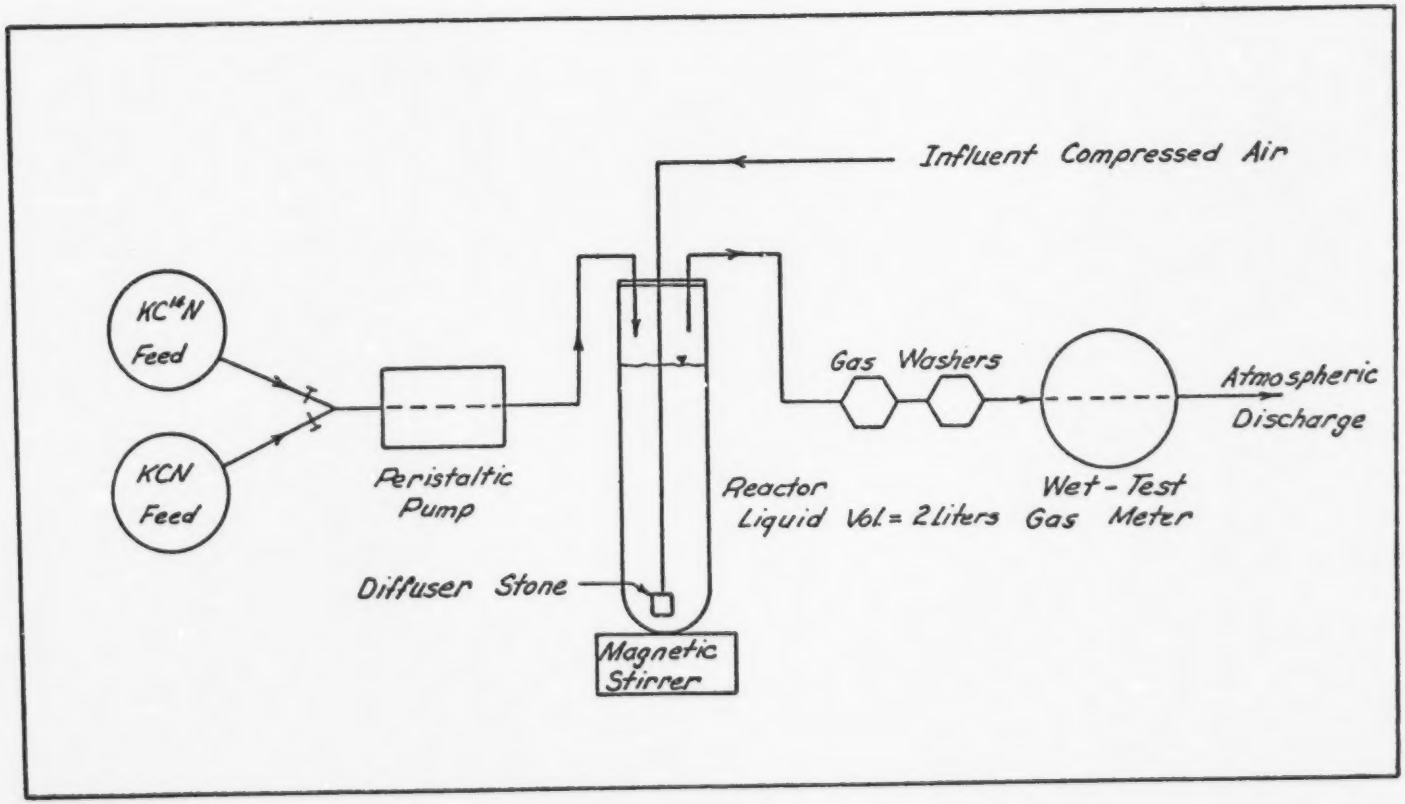


FIG. 14. $KC^{14}N$ study - flow diagram.

the start of the sludge-wasting experiment indicated that the Nitrobacter had not recovered from the biological failure at the end of Phase 3. The nitrite concentrations of the mixed liquor and the effluent decreased steadily for two weeks, at which time they again rose as the ultimate capacity of the system for cyanide was approached.

The appearance of higher concentrations of nitrites near the ultimate loading values of cyanide would be a very important parameter to measure in the operation of a full-scale plant, because it would signal an impending failure and there would be sufficient time to take remedial measures.

7. CYANIDE DEGRADING MECHANISM

The overall mechanism of cyanide degradation in a complete-mixing activated sludge unit with a sewage supplement was investigated by means of radioactive tracer and biochemical studies. The purpose of the tracer experiment was to determine the disposition of the cyanide carbon in a continuously fed unit. The biochemical studies explored the matabolic pathway used by the organisms in the assimilation of the cyanide carbon.

RADIOACTIVE TRACER STUDY

Since cyanide was fed in the form of KCN in all other parts of this research, $KC^{14}N$ was the logical choice for the tracer. Contamination of the pilot plant with the long-lived C-14 would have been detrimental to other radiobiochemical experiments planned for a later date. Consequently, a separate unit of the same type was built for this study. It was smaller and required less of the radioactive material, and was therefore more economical to operate.

EQUIPMENT

The experimental unit, shown schematically in Fig. 14, had two feed reservoirs with identical contents except that part of the cyanide in one reservoir was in the form of $KC^{14}N$. The feed was transferred to the reactor by means of a peristaltic pump.

The reactor was a 3 liter culture tube containing acclimated activated sludge from the pilot plant. Compressed air was admitted through a single diffuser stone located about 1 in. from the bottom of the tube. Exit gases left the top of the reactor through a pair of gas washers filled with sodium hydroxide, then passed through a wet-test gas meter and were discharged into the atmosphere. The top of the tube was sealed with a large rubber stopper to prevent escape of the gases before they passed through the washers. A small magnetic stirrer beneath the reactor ensured good dispersion of the activated sludge, since the diffused gases were considered unreliable for this purpose.

Two gas washers were used in series, the second as a check on the efficiency of the first in removing all carbon dioxide leaving the system.

EXPERIMENTAL PROCEDURE

Acclimated activated sludge from the pilot plant was used in the reactor of the small semicontinuous plant. One liter of sludge was withdrawn from the pilot plant, placed in the reactor, and diluted to 2.0 liters with tap water. The dilution was necessary to prevent the loss of significant quantities of sludge by sampling procedures during the experiment. No more than 1 liter of sludge could be withdrawn from the pilot plant, owing to the danger of overloading the organisms remaining in the tank and causing a biological breakdown.

At the time of the sludge removal, the pilot plant was being fed synthetic sewage and cyanide at a rate of 0.32 l/hr. The cyanide concentration in this feed was 130 mg CN^-/l. The same synthetic sewage was used in the radioactive study, but the feed rate was reduced to one-fifth, or 60 ml/hr, to produce an equivalent loading rate for an equal amount of solids in the smaller unit. The cyanide concentration was also reduced, to 100 mg CN^-/l, to preclude the possibility of incomplete cyanide removals caused by the physical differences of the two systems.

Two identical feed mixtures were formulated and put into separate feed reservoirs. To one of the reservoirs 0.02 millicurie of $KC^{14}N$ was added; the second was left nonradioactive. The 0.02 millicurie of $KC^{14}N$ contributed 0.326 mg of cyanide to the radioactive feed. Since each reservoir contained 1 liter of feed with a cyanide concentration of 100 mg, the radioactive cyanide added to the one reservoir changed the cyanide concentration less than 0.5%, a value insignificant in this experiment.

The system was started at hour 0, at which time a 25 ml sample of the mixed liquor was withdrawn from the reactor to determine the MLSS concentration. The nonradioactive feed solution was applied to the reactor for one hour. The gas washers were then checked qualitatively for the presence of cyanide by the pyridine method (3). No cyanide was present in the washers, an indication that complete cyanide metabolism was taking place in the reactor.

Upon confirmation of efficient cyanide removal, the radioactive cyanide was substituted for the nonradioactive feed and was fed continuously until hour 9. At that time the nonradioactive feed was again applied until hour 23, when feeding was discontinued. The experiment was concluded at hour 29. An 8 hr period of radioactive cyanide feeding was thought to be sufficient for the organisms to reach equilibrium with respect to the tracer material. The system was operated 20 hr longer so that most of the C-14 incorporated in the cells and supernatant could be liberated.

Two objectives were sought in this experiment: first, a quantitative cyanide carbon balance, and second, knowledge of C-14 uptake and release characteristics. Since the carbon could be found only in the mixed liquor and in the effluent gases, these two sources were sampled for assaying throughout the experiment.

The release of carbon dioxide from the metabolism of the cyanide ion was measured by the radioactivity found in the alkaline gas washers.

Every quarter-hour from hour 1.0 to hour 6.5, the alkali in the washers was removed and stored and replaced with fresh base. From hour 6.5 to hour 10.0 the same procedure was performed every half-hour, and then at hours 12.0, 14.0, 16.0, and 29.0.

Mixed-liquor samples were withdrawn from the system each hour until hour 10.0, and thereafter at hours 12.0, 14.0, 16.0, and 29.0. These were 50 ml samples for activity assays. Samples for MLSS determinations were withdrawn from the reactor at hours 0, 5.5, 9.5, and 29.0. Since the system was being fed at the rate of 60 ml/hr, the sludge withdrawn during the first 10 hr of the experiment nearly equaled the volume of feed applied, although the actual solids in the system were reduced during this period from 3.20 g to 2.45 g.

The mixed-liquor samples to be assayed for radioactivity were immediately centrifuged to separate the solids from the surrounding liquid. After centrifugation, the supernatant and solids were put into separate flasks, and a hot ethanol solution was added to them to kill all the organisms present and prevent further metabolism from changing their characteristics. These samples were then stored for the future assay.

The only other measurement taken during the experiment was an hourly reading of the gas flow through the aeration tank. Since carbon dioxide was being trapped, any change of rate during the experiment was important.

ASSAY PROCEDURE

The C-14 fed to the system in the form of the cyanide ion could leave through the exit gas stream as either carbon dioxide or hydrogen cyanide. Qualitative tests on the gas washer samples revealed no loss of cyanide from this source throughout the experiment. The C-14 remaining in the sludge could be in the form of hundreds of different organic metabolic products, either in the supernatant or incorporated in the cells proper.

Barium carbonate was decided upon as the most reliable and most reproducible conversion for the sample counts. All samples were counted under similar conditions. The carbon dioxide in the gas washers presented no problem, as saturated barium chloride precipitated all the carbonate as barium carbonate immediately. The organic carbon in the centrifuged mixed-liquor solids and in the supernatant was first converted to carbon dioxide, then trapped in sodium hydroxide, and finally converted to barium carbonate.

Initially, the conversion was made with a modified Van Slyke reagent consisting of a 10:1 solid mixture of KIO_3 and $K_2Cr_2O_7$ combined with a 1:1 liquid mixture of H_2SO_4 and H_3PO_4 in the combustion vessel. A 4N $K_2Cr_2O_7$ and H_2SO_4 mixture in a reflux apparatus proved to be as effective, and these reagents were used for most of the combustions, which were carried out with a 30 min reflux time.

The combustion train consisted of a 500 ml three-necked distillation flask with a 250 ml separatory funnel, containing the sulfuric acid, fitted

to one neck. A valve was placed in the center neck so that the air from outside the flask could be cut off. A condenser, 300 ml in length, was connected to the last neck. A valve connected to the top of this condenser permitted the contents of the flask to be discharged either to the atmosphere or to the line that carried the stream through a gas washer. All joints were 24/40 standard taper except along the line leading to the gas washer, where 18/9 ball joints were used.

The sample was placed in the distillation flask with a few glass beads to prevent bumping, and the dichromate solution was added. The system was closed, and a vacuum was applied to the exit side of the gas washer until a partial vacuum was created in the system. Then the center valve on the flask and the valve on the funnel containing the acid were opened. The vacuum had to be sufficient to ensure that all of the gases evolving from the acid-dichromate mixture went through the train and not out of the flask. Heat was applied with a small Bunsen burner, and the mixture was refluxed for 30 min. After cooling, the sodium hydroxide in the gas washer was assayed for radioactivity in the usual manner.

The presence of alcohol in the samples necessitated a very large quantity of the wet combustion mixture, because the alcohol had to be combusted to carbon dioxide and water to make certain that the other organic material was combusted. To conserve chemicals, as well as to prevent undue dilution of the carbonate, the ethanol was removed first from both supernatant and solids by adding water, pH 11, to the samples and distilling off the ethanol.

An aliquot of the sodium hydroxide from the combustion or gas washer samples was then treated to produce the barium carbonate that was to be plated.

The cyanide feed solution also had to be converted to barium carbonate to provide a sample for calculating the total amount of active material fed to the system during the experiment. The cyanide feed was made alkaline with sodium hydroxide, and chlorine gas was bubbled through the solution until the pH was lowered to 10.0 from its original value of 12.5. The resulting solution was tested for cyanide by the pyridine method to make sure that all of the cyanide had been converted to carbon dioxide. A sample of this solution was then plated in the normal manner.

Reiger (39), in a study of barium carbonate plating procedures, found that slow crystal growth produces the best crystals for this type of work. For slow growth, the following conditions should be met: dilute reagents, elevated temperature, and lowered alkalinity. Reiger accomplished the last condition by adding ammonium chloride to the sodium hydroxide.

Infinitely thick plates of barium carbonate were used in this work rather than absolute counts, because with this thickness the count is directly proportional to the specific activity of the barium carbonate. An infinitely thick plate of barium carbonate has a thickness of 20 mg/sq cm (6). Thick plates have the disadvantages that they unduly dilute small samples, that the barium carbonate tends to form fissures on drying, and that the observed disintegrations must be corrected for thickness, which

requires accurate weighing. These disadvantages are outweighed by the fact that all of the compounds present can be made into a carbonate, thus giving the uniformity needed for this assay.

In most cases a 50 ml aliquot was taken from the 200 ml total volume of the gas washers and transferred to a 100 ml beaker. To the beaker was added 15 ml of a 4N ammonium chloride solution, and 10 ml of a potassium carbonate solution containing enough carbonate to produce approximately 100 mg of barium carbonate upon precipitation. This mixture was stirred and heated to near boiling. When it was removed from the burner, 2 ml of saturated barium chloride were added. Precipitation of the carbonate was immediate. The flask was then placed in a water bath to cool to room temperature, and the carbonate was subsequently plated.

A modification of the plating technique described by Overman and Clark (31) was used throughout the assay procedure. About 30 holes were drilled with a 3/32 in. drill in the bottom of a 1.5 cm by 0.5 cm cupped aluminum planchet. A piece of No. 42 filter paper was cut to a slightly smaller diameter than the inside of the planchet and placed in the cup. All planchets were numbered, dried, and tared before use.

The tared planchet and filter paper were placed in a filter holder on a vacuum filtration flask. A small vacuum was applied, and the cooled supernatant and precipitate were filtered through the paper. After all the material had been filtered, the carbonate was thoroughly washed with 100% ethanol to ensure that hard granular crystals would form on the plate.

The wet plates were placed under infrared lamps to dry for a minimum of 20 min, cooled in a desiccator, and reweighed. The 20 min infrared drying gave the same weight as a 2 hr oven drying at 103° C, and was therefore chosen to save time.

A thin-window Geiger counter equipped with an automatic sample changer and print-out interval timer was used for counting all samples. Background was determined with a barium carbonate plate made from the potassium carbonate used for the dilution of the samples. The background with this plate was 18.2 ± 0.34 counts per minute (cpm).

EXIT GAS RESULTS

The exit gases were sampled by the procedure just described. Figure 15 shows the activity encountered above background in the gas washers. These values have all been corrected for the gas rate through the reactor, the aliquot of the total volume of absorbing alkali, the period of exposure of the alkali, the background, and the thickness of the final plate. The upper curve is expressed in counts per minute per minute of exposure of the alkali, per cubic foot per hour of air passed through the reactor. The lower half of Fig. 15 shows the total carbon dioxide leaving the reactor, expressed as a percentage of the maximum carbon dioxide evolution per unit time. The maximum carbon dioxide release was at hour 5. All points are plotted at the central time of sampling. Thus the sample collected for 2 hr starting at hour 14 and ending at hour 16 was plotted at hour 15.

Activity was detected in the exit gases within 15 min of the starting time of the radioactive application, an indication that one of the metabolic reactions took place rapidly. The activity increased to a very sharp peak at hour 5, then dropped back to a plateau about hour 6. The total amount of carbon expired also rose at hour 5 and subsequently dropped back to its former level. The high peak was considered to be a function not of the metabolism but of a chemical-physical change occurring in the reactor. A small temperature rise in the reactor caused by heat from the magnetic stirrer motor might have been sufficient to alter the carbonate equilibrium conditions, thus releasing carbon dioxide. A pH change in the reactor could very well have produced the same effect. It must be assumed that equilibrium conditions had not been attained with respect to $C^{14}O_2$ until about hour 5.5 at the earliest. A similar explanation can be made for the very high point plotted at hour 11, since an unusually high total carbon dioxide value also was found at this time.

Assuming that the plateau would have started at hour 5 if it had not been influenced externally, the organisms took at least four hours to reach equilibrium. This indicated that the overall metabolic reaction within the reactor was quite slow and was probably not wholly a function of respiration.

Figure 16 is a summation of the corrected gas washer counts for the period from hour 5.5 to 9.0. The straight line shows that equilibrium had been reached with respect to radioactive carbon dioxide evolution, which in turn indicates equilibrium conditions for cyanide metabolism. The slope of one-third of the counts applied to the system in the feed during the same period is also plotted in Fig. 16. McKinney (26) stated that one-third of the ultimate oxygen demand of the material being metabolized is oxidized and the remaining two-thirds is converted to protoplasm. The carbon dioxide recovery data from the radiotracer study confirm McKinney's finding, as can be seen in the slopes of the two curves shown. The recovered portion had a slope of 1.37, and one-third of the feed C-14 is 1.42.

MIXED-LIQUOR ASSAY RESULTS

Figure 17 depicts the relative proportion of counts in the mixed liquor at all sampling times throughout the experiment. The supernatant and solids fractions of the mixed liquor are also shown. The relatively complicated treatment used to convert the two fractions to barium carbonate, as well as the sample size in relation to the total volume in the reactor, caused erratic results.

The increasing activity in the solids was less than that of the supernatant until about hour 8, at which time the solids reached a very rough plateau. The supernatant value for hour 9 was extremely high, causing a high total value for the mixed-liquor activity. A second aliquot of the sodium hydroxide was tried, with the same result. Unless contaminated glassware was used in the combustion procedure, this high value must be considered a valid datum.

The supernatant activity steadily decreased from hour 9 to the end of the experiment, at which time very little activity was present. The

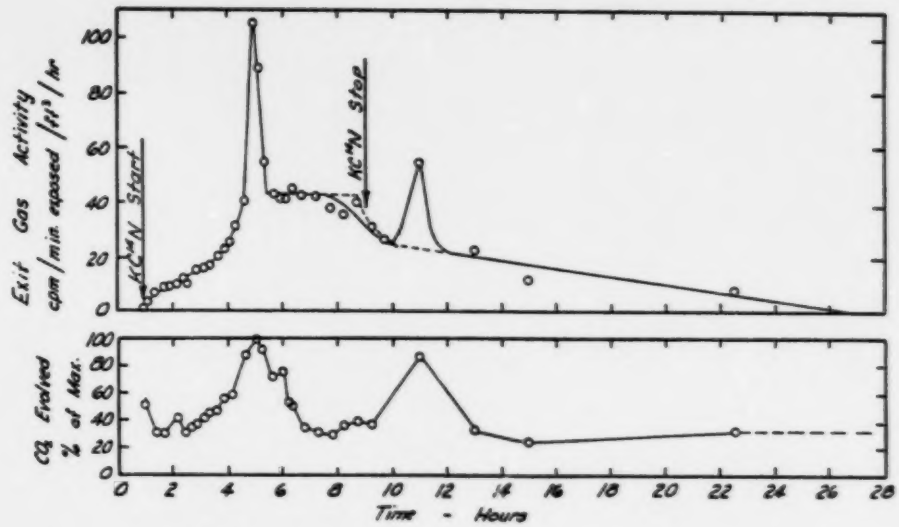


FIG. 15. $KC^{14}N$ study - exit gas activity and relative CO_2 release.

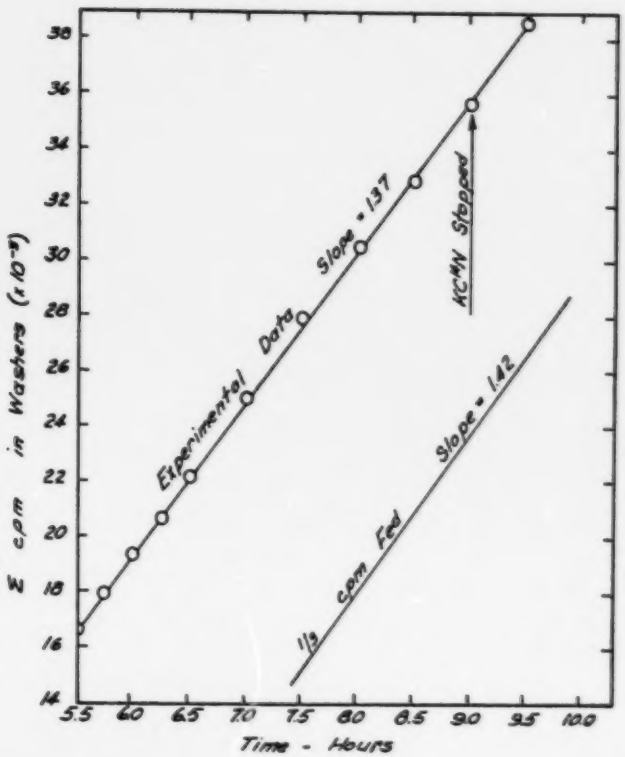


FIG. 16. $KC^{14}N$ study - counts per minute in exit gases during equilibrium.

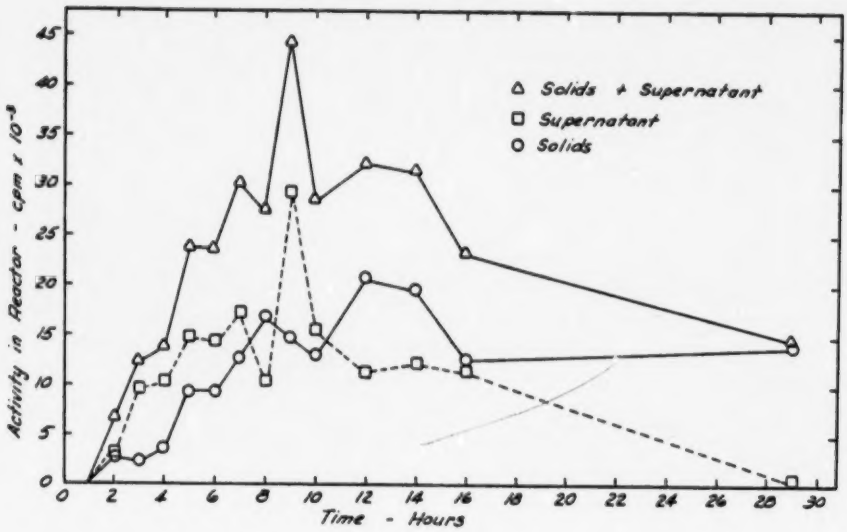


FIG. 17. $KC^{14}N$ study - instantaneous reactor activity.

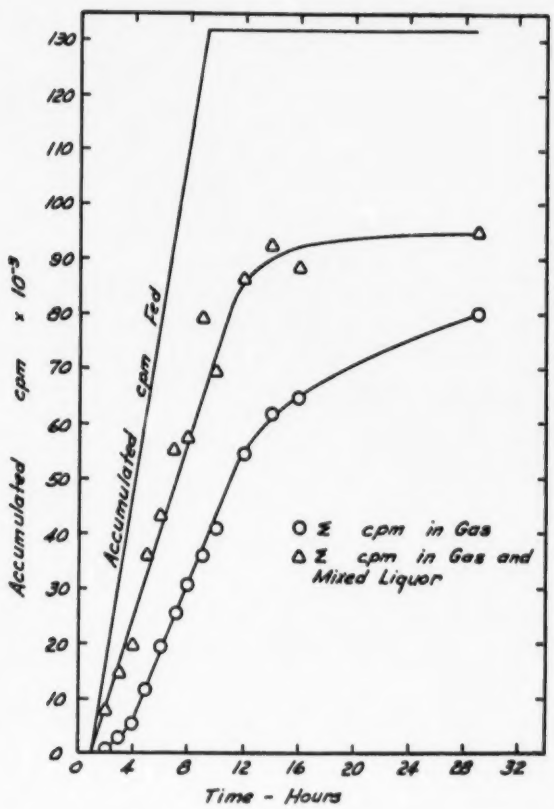


FIG. 18. $KC^{14}N$ study - carbon-14 recovery.

activity of the solids stayed above that of the supernatant for the remainder of the experiment, the final activity being about equal to the rough plateau between hours 10 and 16. The mere fact that significant activity was found in the cells 21 hr after C-14 additions had ceased is qualitative proof that the organisms present used the cyanide carbon for cell synthesis.

The activity in the mixed-liquor supernatant decreased about 12,000 cpm from hour 16 to hour 29, but 16,000 cpm were recovered in the exit gas during the same period. Since the activity in the cells was relatively constant, the 4000 cpm difference must have been caused by experimental error. The supernatant samples were subjected to many more manipulations and chemical reactions than were the gas samples. It must be concluded that neither the supernatant nor the solids samples were quantitatively converted to carbon dioxide and water.

Figure 18 shows the summation curves for activity applied, total activity recovered, and activity recovered in the exit gases. The sum of the mixed-liquor fraction at any time, plus the accumulated counts per minute recovered in the gas up to that time, should be equal to the total activity fed to the organisms to that time unless 100% recovery was not attained. A total activity of 131,000 cpm was fed to the system, and 95,000 cpm were recovered in all samples by the end of the 29-hour experiment. Hence overall recovery for the experiment was 72.5%. Most of the counts recovered at the end of the experiment were from the gases. If the experiment had run for a greater length of time, so that the turnover rate of the organism was exceeded, nearly all of the C-14 would have been recovered in the exit gas washers as carbon dioxide. The data from this experiment do not reveal the turnover rate.

At no time did the total recovered counts per minute equal the counts fed. The overall results must be considered qualitative, although the results of the assay of the exit gases are thought to be more accurate than the data as a whole.

BIOCHEMICAL STUDIES

In an attempt to gain knowledge of the metabolic pathway used in cyanide degradation, Miller* performed studies using the acclimated culture from the pilot plant. The studies were carried out in stoppered flasks fitted with a hanging center well containing a solution of 0.88M KCN and 0.5M KOH that absorbed any carbon dioxide or hydrogen cyanide lost from the cell solution. Various quantities of labeled potassium cyanide were mixed with the synthetic sewage and applied to the flasks, which contained a known weight of activated sludge. The flasks were shaken mechanically to ensure diffusion of the gases through the liquid.

At the end of the exposure time, which varied from 5 to 60 min, the cells were killed with a quantity of boiling absolute ethanol that gave

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*A. P. Miller, unpublished data, 1963, Department of Biochemistry, The Pennsylvania State University.

a final ethanol-water dilution of 80:20. A water extraction of this residue was then performed with distilled water, pH 10.6. The final residues were hydrolyzed by boiling with 12.5N NaOH for 30 min in a hot water bath. Ethanol and water extractions usually contain compounds of low molecular weight, such as amino acids, simple organic acids, and sugars. Those of higher molecular weight, such as proteins and polysaccharides, are normally found in the residue from this type of procedure.

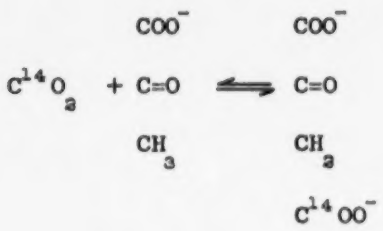
The various extractions and residues were dried on planchets and assayed with the Geiger counter used in the tracer experiment. Most of the activity of this qualitative study was found in the ethanol extraction, with only small quantities in the center wells. The various fractions were tested for $KC^{14}N$, but none was detected. Because the pH of the cultures was usually above 8.6 even after 60 min exposure, determination of the amount of radioactive carbonate in the various extractions was deemed necessary. The results showed that 76% of the ethanol extractions, 88% of the residue, and variable percentages of the distilled water fractions were in the form of carbonates. Since not all of the activity was found to be in the form of a carbonate, it has to be assumed that the remainder of the C-14 was metabolized and incorporated into the cellular material of the organisms.

Two-dimensional paper chromatography was used to separate the compounds from the ethanol and water extractions, since these compounds were likely to have low molecular weight. A small portion of an extraction was spotted on one corner of a sheet of No. 1 paper, and an 80:20 phenol-water solvent was then allowed to run the paper lengthwise. After drying, the paper was run across the width with a 80:20 propanol-water solvent in an ammonia atmosphere and dried again. It was then placed in a film holder against a sheet of X-ray film, which was exposed for approximately one month. The long exposure was necessitated by the low activity level of the spots on the chromatogram.

The developed radiograms revealed many small spots and five large dark areas. Because of time limits, identification of only the larger spots was undertaken. Various indicator sprays were applied to the papers in an attempt to chemically classify the compounds present. Although this method by no means identifies a chemical group absolutely, it does serve as a starting point for further analysis.

Based upon R_f values, co-chromatographing with suspect amino acids, and paper electrophoresis, the first major labeled compound was tentatively identified as glutamic acid. This acid was the only acid present from the 20 to 60 min exposures in the ethanol extractions, but two other amino acids were found in extracts from 5 min exposures. Time did not permit the identification of any other labeled compounds.

The tentative indentification of glutamic acid offers some evidence for carbon dioxide fixation, probably as observed by Wood and Werkman (49):



pyruvate oxaloacetate

Since oxaloacetate is an intermediate in the citric acid cycle, other members of this cycle as well as reaction products of it should be found labeled. If this were the case, glutamate could be formed via a transamination of alpha-ketogluterate, another intermediate of this cycle, and the label would be in the fifth carbon of the glutamate.

In conclusion, part of the cyanide carbon fed the culture was definitely found incorporated in the cell material, probably by means of the citric acid cycle. Carbon dioxide fixation is believed to be the primary entry into this cycle.

PILOT PLANT MECHANISM

The results of the pilot plant studies, the radioactive tracer experiment, and the biochemical studies have all contributed to a better understanding of the biological mechanism in activated sludge assimilation of the cyanide ion.

Phase 3 of the extended aeration study, the radioactive tracer study, and the biochemical studies proved that part of the cyanide carbon was used for cell synthesis. The solids balance of Phase 3 indicated that two-thirds of this carbon went directly into new cells. The tracer study showed that one-third went into the mixed liquor, one-third to respiration, and approximately one-third was unaccounted for in the assay. The biochemical studies definitely showed that the carbon became involved in the metabolism of the cells, probably through the citric acid cycle. If the carbon not accounted for in the tracer study was incorporated into the cellular material, then two-thirds of the cyanide carbon was used for cell synthesis. The overall plant mechanism is therefore considered to function on the one-third/two-thirds relationship with respect to the cyanide carbon.

No quantitative data were gathered for the oxidation-synthesis relationships of the synthetic sewage, but the same proportions can be assumed as those determined for the cyanide carbon.

The cyanide nitrogen was converted quantitatively to nitrate nitrogen in all experiments performed with the synthetic sewage supplement, except under the adverse conditions of cyanide overloads. That the

organic nitrogen contributed by the synthetic sewage was partially converted to nitrate nitrogen and partially converted to cell protoplasm was indicated by the nitrogen balances performed during the equilibrium period when no cyanide was in the feed. Since the ratio of these two sources of nitrogen recovery was variable, depending upon the rate of synthesis in the system at any given time, a precise ratio for the ultimate form of the nitrogen in the mixed liquor was impossible to determine.

The other major components of the synthetic sewage were biologically degraded and are assumed to have been incorporated in the cells, completely oxidized, or discharged in the effluent.

8. PLANT DESIGN FACTORS AND COST COMPARISON

The bench-scale pilot plant studies described in the preceding sections showed that the cyanide ion is amenable to complete-mixing activated sludge treatment with sewage as the supplemental feed. The advantage of biological cyanide degradation should be its economy as compared with alkaline chlorination, the prevailing chemical method. To measure this advantage, design factors for a full-scale prototype plant were projected from the research data, and the estimated installation and operating costs for the biological plant were compared with corresponding costs for chemical treatment of the same amount of cyanide.

PLANT DESIGN FACTORS

From the following discussion it will be apparent that the design factors for a particular installation would depend on the number of employees, the number of shifts worked, the quantity and strength of the sewage, the quantity and strength of the cyanide waste, and the location of the nearest domestic sewer.

The experimental results were extrapolated to a full-scale plant by calculating the treatment of a unit weight of cyanide per unit time. Sludge wasting was chosen as the operating practice for the prototype plant, to prevent uncontrolled loss of the mixed liquor and consequent breakdowns due to overdosing the organisms in the aeration tank with cyanide. For these calculations, it was decided that the mixed-liquor solids concentration would be held fairly constant at 2000 mg/l, with 85% volatile solids. The detention time in the aeration tank was set at 22 hr, the value used during the sludge-wasting experiment.

To treat 1 lb of cyanide per hour at a loading rate of 3.5 mg CN^-/g MLVS/hr, the loading recommended in Section 6, the aeration tank must contain 337 lb MLSS. At 2000 mg MLSS/l, the aeration capacity needed per hour of detention time is 122 cu ft, or 910 gal. If the concentration of cyanide waste is 400 mg/l, the flow from this source to provide 1 lb of cyanide would be 40 cu ft/hr, or 300 gal/hr. The organic load needed for proper operation of the treatment unit would therefore have to be contributed by a domestic sewage flow of 610 gal/hr.

From light manufacturing, the domestic flow is 25 gal per person per shift, with a BOD of 0.06 lb per person per shift (27). More complex manufacturing operations produce 40 gal per person, with a BOD of 0.10 lb. At an average value of 32 gal per person per shift and 0.08 lb BOD per person per shift, the total number of employees needed to provide a flow of 610 gal/hr is 455.

For a self-sustained system with cyanide waste discharging into the aeration tank uniformly over a 24 hr period, a storage tank for the domestic flow would be required. An alternative procedure would be to keep the domestic flow uniform by pumping supplementary sewage to the treatment plant from a nearby municipal sewer. Although the strength of this sewage would be quite weak during the night hours, the storage tank and its aeration facilities would be unnecessary.

Sewage from an area that is essentially residential with some business and light industry is rated at 100 gal per person per day, with a BOD of 0.2 lb per person per day or about 240 mg/l. Used as supplementary feed, it would reduce the employee population required for a flow of 610 gal/hr to only 144 persons, a value so low that operation of a treatment plant should be feasible in almost any populated area.

COST COMPARISON

GENERAL ASSUMPTIONS

Location. Since most industries that produce cyanide wastes are found in or near metropolitan areas, the hypothetical industry on which the economic study is based is assumed to be located in such an area. A municipal sewer is therefore available to receive the effluent from the treatment processes and the intentionally wasted sludge from the biological treatment plant, eliminating the need for special sludge-handling and disposal facilities. The sewer is also used as a source of additional domestic sewage for the prototype plant.

Cyanide Waste. Table 5 gives the assumed characteristics of the cyanide waste from this industry. These are the functional limits for the cost comparison of the biological and chemical treatment methods.

TABLE 5. Waste Characteristics, Prototype Plant

Waste flow per year	2,500,000 gal
Working days per year (1 shift)	255 days
Waste flow per working day	9,800 gal
Waste flow per treatment day*	6,850 gal
Cyanide concentration, avg	380 mg/l
Cyanide applied per day	21.8 lb
Cyanide applied per hour	0.91 lb

*Plant operates 7 days per week.

It should be noted that the economic study considers only those costs directly related to the removal of the cyanide ion, ignoring other components of the waste that might require treatment.

Land Costs. Land costs are not included in the comparison, because the company would use about the same amount of land for either method. The land costs are assumed to cancel out, and would therefore have no effect on the unit costs for treatment.

Man Costs. Salaries and wages for the two types of treatment plants are also assumed to be about equal, and are likewise omitted from the comparison. Since the chemical process can be geared to the normal work week, the chlorination plant would be in operation fewer hours than the biological unit, which must work continuously. But the chemical plant requires more personnel, because the operator does not have time for all the upkeep and minor repairs. The combined man costs for one skilled operator and one unskilled assistant for the chemical plant are estimated to be about $12,000 per year.

The biological plant could be operated by one person, except that it must function 24 hours per day, 7 days a week. Someone must check its efficiency periodically when the operator is not present. The main concern would be a possible biological failure, which would be forecast by the appearance of cyanide in the effluent. A watchman could easily be trained to add prepared detector solutions to an effluent sample, observe a color signal, and notify the plant operator of impending trouble. The salary of the operator and the portion of the watchman's wages chargeable to time spent on testing duties are estimated at $12,000 per year, or about the same total as the man costs for chemical treatment.

Because full-scale industrial treatment of cyanide by the biological method is new and untried, the prototype plant would require significantly more attention from trained personnel during its first months. After a few of these plants have been built and are working, the start-up and operating problems should be minor. The cost comparison assumes an established plant, and therefore does not include the abnormal man costs that would undoubtedly be incurred in launching the first full-scale installation.

BIOLOGICAL TREATMENT

Installation and operating costs for the biological treatment plant are based on the plant design factors previously discussed and the cyanide waste characteristics given in Table 5.

The cyanide waste from the industry flows by gravity into a holding tank large enough to retain a two-day accumulation of waste, or approximately 20,000 gal. From the holding tank the waste is bled into the nearby aeration tank over a 24 hr period, 7 days a week, by means of a pump. This operation must be performed in such a way that the tank is nearly empty on Monday morning and nearly full on Friday night, so that the waste supply is sufficient to carry the unit over the week end. Although the plant can be "starved" for short periods, this practice is not advisable with a waste as toxic as cyanide.

The plant is designed for 2000 mg MLSS/l with 85% volatile solids, a cyanide loading rate of 3.5 mg CN^-/g MLVS/hr, and 22 hr detention time. The volume of waste to be put into the aeration tank each day is 6850 gal (Table 5). According to the design parameters, to treat 0.91 lb CN^-/hr the capacity of the aeration tank would be calculated as follows: (0.91 lb CN^- per hr) × (122 cu ft per hr detention time/lb CN^- per hr) × (22 hr detention time) = 2440 cu ft, or 18,300 gal. The daily flow of waste plus domestic sewage would therefore be 18,300 gal × (24 hr per day/22 hr detention time) = 20,000 gal.

McKinney (50) states that the installed cost of a prebuilt activated sludge treatment unit is about $1.50 per gallon of waste treated per day -- in this case $1.50 × 20,000 gal, or $30,000.

Two pump units are needed. One constantly pumps the cyanide waste from the holding tank into the aeration tank. The other brings domestic sewage from the sewer to the aeration tank during periods when the domestic sewage flow from the plant is too low to contribute the supplemental feed at the required rate of 13,150 gal per day (20,000 gal less 6850 gal).

Table 6 gives estimated initial costs and annual operating costs for the biological treatment plant, excluding the land and man costs previously discussed, and the cost of treating 1 lb of cyanide. Since the plant is to treat 7930 lb of cyanide per year, the unit costs were obtained by dividing the annual costs by this weight. It should be noted that the installation requires no building.

TABLE 6. Cost Summary - Biological Cyanide Treatment

Item	Initial Cost	Annual Cost	Cost/lb CN^-
Installation			
Holding tank	$ 4,000 [40]*	$ 100	$ 0.013
Bio-plant (prebuilt)	30,000 [40]	750	0.094
Pump units (2)	7,000 [10]	700	0.088
Pipe from sewer	1,000 [40]	25	0.003
Pipe to sewer	1,000 [40]	25	0.003
		Subtotal	$ 0.201
Operation			
Power, compressed air		$1200	$ 0.152
Power, pumping		500	0.063
Maintenance supplies		2500	0.316
		Subtotal	$ 0.531
		Overall Total	$ 0.73

*Bracketed numbers are amortization period, years.

Assuming an established plant, the estimated total cost of degrading industrial cyanide waste by the biological method is $0.73 per pound of cyanide treated.

ALKALINE CHLORINATION

In an industry working one shift per day, cyanide treatment by alkaline chlorination is a batch operation. Three reaction tanks are needed, each capable of holding the cyanide waste from a single day. The tanks are used in continuous sequence. While one is collecting the day's waste, the solution collected in another on the previous day is adjusted for pH, chlorinated, and mixed. The chlorinated mixture is held in the tank for about 20 hr to ensure complete conversion of the cyanide to carbon dioxide and nitrogen gas, and is then dechlorinated and discharged into the sewer. While this tank is being refilled, the wastes in the first tank are treated and those in the third tank are dechlorinated and discharged into the sewer.

This plant requires a building for equipment housing and chemical storage. The equipment includes a chlorinator, a chemical feeder, one pump unit for the raw waste, three mixing units, and an outfall line to the sewer.

Table 7 is the cost analysis for the chemical method, based on the assumptions of Table 5. As in Table 6, the unit costs were found by dividing the annual costs by 7930 lb, the weight of cyanide to be treated per year. The estimated total cost of treating industrial cyanide waste by alkaline chlorination is $1.44 per pound of cyanide treated.

TABLE 7. Cost Summary - Chemical Cyanide Treatment

Item	Initial Cost	Annual Cost	Cost/lb CN^-
Installation			
Reaction tanks (3)	$ 6,000 [40]*	$ 150	$ 0.019
Chlorinator	4,000 [10]	400	0.051
Chemical feeder	5,000 [10]	500	0.063
Mixing units (3)	3,000 [10]	300	0.038
Pump unit	3,200 [10]	320	0.040
Building	15,000 [40]	375	0.047
Pipe to sewer	1,000 [40]	25	0.003
		Subtotal	$ 0.261
Operation			
Chemicals		$6350	$ 0.800**
Power		500	0.063
Maintenance supplies		2500	0.316
		Subtotal	$ 1.179
		Overall Total	$ 1.44

*Bracketed numbers are amortization period, years.

**Cost figure obtained from plant now practicing alkaline chlorination.

DISCUSSION

On the evidence of Tables 6 and 7, the unit cost of treating cyanide biologically is about half the cost of alkaline chlorination. The biological method is therefore worthy of further consideration as a means of degrading industrial cyanide wastes.

Besides its economy, biological treatment has another advantage. If the operations of the industry should expand during the life of its treatment plant, the quantity of waste would probably increase proportionately. An alkaline chlorination plant would have to be expanded to handle a larger volume of waste, and the cost of the chemicals would rise in direct proportion to the increased load. The only change necessary for a biological plant would be a larger holding tank. The aeration tank could carry a higher mixed-liquor solids concentration and would thus be able to treat a greater weight of cyanide, since the design basis was weight of cyanide per weight of solids per unit time. Operating costs would be somewhat higher because of the increased oxygen demand of the additional solids in the aeration tank, but the rise would not be proportional to the increased volume of waste as it would in the chemical process.

9. CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

The following conclusions may be drawn from the experimental studies and the economic analysis.

(1) With a supplemental feed of domestic sewage, an acclimated complete-mixing activated sludge unit is capable of degrading the cyanide ion at a rate adequate for the treatment of cyanide in industrial wastes. The system can degrade 99% or more of the cyanide ion at cyanide loading rates as high as 5 mg CN^-/g MLVS/hr.

(2) Though solids age does not appear to influence the rate of cyanide removal, intentional sludge wasting (young cells) is preferable to extended aeration (old cells). Sludge wasting prevents the biological failure that tends to occur when cyanide loads are held constant under extended aeration. It also permits the system to handle shock loads as great as twice the existing cyanide loading without failure.

(3) The radioactive tracer and biochemical studies confirmed (a) that under equilibrium conditions one-third of the cyanide carbon is used by the biota for respiration, and (b) that two-thirds of the cyanide carbon is converted to cellular material, probably entering the metabolic system through the citric acid cycle.

(4) Since the cyanide nitrogen is converted quantitatively to nitrate nitrogen at all times except immediately before and during a biological failure, the appearance of ammonia and nitrite nitrogen in the effluent from a working plant would be a useful trouble signal.

(5) In an established plant, the cost of biological treatment of industrial cyanide waste should be approximately half the cost of chemical treatment by alkaline chlorination.

(6) The complete-mixing activated sludge process, as here applied, is a feasible and economical means of degrading the cyanide ion in industrial cyanide wastes.

RECOMMENDATIONS

Observations during the research here reported suggest further investigation in several areas.

(1) To determine the optimum age of cells for efficient cyanide removal, the effect of solids ages different from those used in these studies should be examined.

(2) The minimum supplemental feed necessary to treat a unit weight of cyanide should be calculated. Because many cyanide wastes have low cyanide concentrations and would therefore dilute the supplement appreciably, this may be an important parameter in designing the size of the aeration tank for some installations.

(3) It would be useful to know whether the presence of ammonia or the lack of organic trace elements caused poor cyanide metabolism with the ammonium acetate supplement.

(4) Since nearly all cyanide wastes contain heavy metals, the effect of heavy metal ions on treatment efficiency should be studied.

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